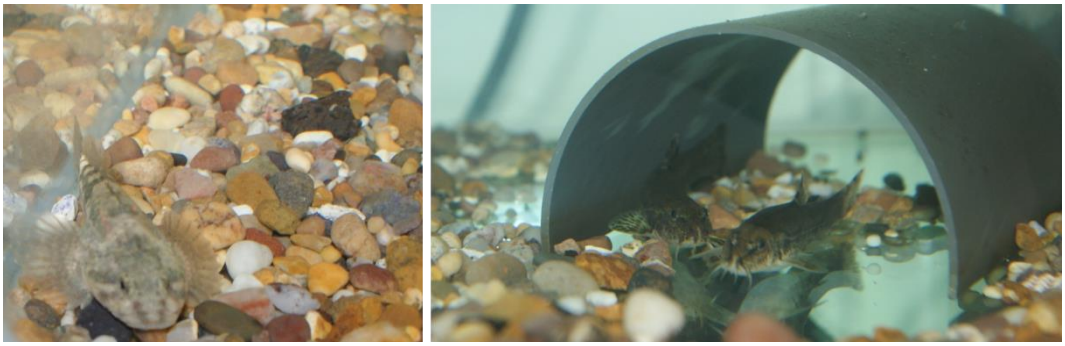
# Invasion Physiology: Do physiological characteristics facilitate the spread of invasive fish species?



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# Abstract

A considerable number of abiotic (e.g. temperature) and biotic factors (e.g. intra-interspecific interactions) contribute in shaping species' distribution and invasiveness but knowledge is still lacking regarding the importance of physiological and behavioural traits in determining the distributions of ectotherms and especially the invasion of non-native species into novel habitats. With rising temperatures, distribution shifts in many of fish species have been observed. Additionally, changing thermal conditions are facilitating the colonisation of invasive species. It is crucial that we gain an increased understanding of the mechanisms underlying the effects of environmental change on the distribution of aquatic species and the ecological damage caused by invasive species.

Physiological traits are likely to present a fundamental constraint on the environments that are habitable to a given species. Whole animal traits associated with energy metabolism and locomotory performance are especially likely to be important in this regard. Nevertheless, the effects of traits such as metabolic rate and aerobic scope (AS) on the distributions of native and invasive species have not been thoroughly studied. In a first step toward understanding these effects, I performed a phylogenetically-informed analysis of links between AS and absolute latitudinal distribution range in 86 fish species. From the results obtained in Chapter 2, I found no evidence for the direct implication of AS in the currently observed distributions of fishes. Moreover, no association between AS and invasion success in 59 freshwater fish species (23 invasive and 36 native species) was found. These results suggested that peak AS is not a constraining or determining factor in the distribution range and invasion success in fish.

Following on from these results, there is a possibility that there could be a trade-off between peak and breadth of performance for AS across temperatures in fishes. Species with a higher peak AS might only be able to function normally over a narrow range of temperatures. In Chapter 3, I collected AS data from literature and conducted phylogenetical-informed analysis to test the trade-off theory in AS across 28 fish species. No evidence could be found for a trade-off between peak and breadth performance in AS for fish.

Interspecific competition between invasive and native fish species might cause changes in the structure of native fish communities. Furthermore, these interactions can vary over competitive context (e.g. for prey or cover), differ over a range of environmental factors (e.g. in response to temperature variation) and be linked directly or indirectly to species' metabolic capacity (e.g. aerobic scope). With increasing temperatures, invasive species might gain a competitive advantage over the native species though shifts or changes in competitive behaviour and traits such as AS. After having investigated broad patterns among AS and geographical distributions in fishes, the remainder of my thesis focused on trade-offs in energy allocation and tolerance to environmental stressors in a pair of species to determine the role of aerobic capacity as a factor in competition between these two species. Specifically, I examined interactions between native stone loaches (*Barbatula barbatula*) and invasive bullheads (*Cottus gobio*), two species which occupy the same ecological niche and that are believed to compete for similar habitats. Physiological and behavioural traits could play an essential role in the spread of invasive species, particularly the internal underlying mechanisms that modulate an organism's response to environmental changes. In Chapter 4, I examined physiological and behavioural responses of invasive bullheads and native stone loaches to acute and acclimated temperature shifts (13-21°). I found that invasive bullheads had a lower AS than stone loaches over all temperatures tested. Bullheads were also less active overall and preferred colder temperatures (17.5-19°C) than stone loaches (21-22.4°C). Therefore, changes in AS in response to thermal variation are unlikely to be a contributing factor in invasion success of bullheads in Scottish rivers.

In Chapter 5, I investigated the direct competitive interactions between bullheads and stone loaches at three different temperatures (13°C, 17°C and 21°C). Overall, native stone loaches were better competitors for shelter use and in particular at colder temperatures. There was no clear causal effect of temperature or AS on competitive outcomes between these two species. Low competitive ability found in invasive bullheads suggests that bullheads may not be actively displacing stone loaches.

It has been suggested that a successful invader should have a wide tolerance range for different environmental factors. For example, invasive species might be more tolerant to hypoxic events as compared to native species. In Chapter 6, I looked at the hypoxia tolerance and avoidance behaviour of bullheads and stone loaches over different dissolved oxygen (DO) concentrations (100%, 80%, 60%, 40%, 30%, 25% and 20% DO levels).

Surprisingly, I found that bullheads were less tolerant to hypoxic conditions with a $P_{crit}$ value of 4.96 mg $O_2$ $l^{-1}$ at 14°C. Avoidance behaviour towards progressive hypoxia was similar between bullheads and stone loaches; both species spent most of their time utilising the shelter even in hypoxic conditions. Low tolerance towards hypoxia did not give an advantage to invasive bullheads over native stone loaches in particular during harsh environmental conditions.

The results from this thesis suggest that metabolic traits may not play as strong a role in constraining species distributions as previously suspected, particularly in the specific case of interactions between native stone loaches and invasive bullheads in Scotland. Instead, other physiological factors, life history traits, and population demographics may play a primary role in affecting invasion success in this case.

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# Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Signature _____

Printed name _____

# Abbreviations

**AS**: Aerobic Scope

**BMR**: Basal Metabolic Rate

**CI**: Confidence Interval

**CTmax**: Upper thermal tolerance limits temperature

**CTmin**: Lower thermal tolerance limits temperature

**DF**: Degrees of Freedom

**DO**: Dissolved Oxygen levels

**GLM**: Generalised Linear Model

**GLMM**: Generalised Linear Mixed Model

**MMR**: Maximum Metabolic Rate

**$MO_2$**: Oxygen uptake rate

**$P_{crit}$**: Critical oxygen partial pressure point

**PGLS**: Phylogenetic Generalised Least Squares model

**$P_{max}$**: Peak Aerobic Scope

**RMR**: Resting Metabolic Rate

**SEM**: Standard Error

**SMR**: Standard Metabolic Rate

**$T_{breadth}$**: Thermal breadth performance

**$T_{crit}$**: Critical Temperature

**$T_{opt}$**: Optimal Temperature

**TPC**: Thermal Performance Curve

# Chapter 1: Introduction

## 1.1 Physiology and distribution trends in animals

Alexander von Humboldt (1769–1859) first described the biogeographical trends observed in fauna and flora across the world. Since this time, ecologists have been interested in the factors which limit and determine species distribution ranges on Earth. The geographical distribution of species can span from broad ranges for cosmopolitan species to endemic species only found at one particular location. Several abiotic (e.g. environmental temperature) and biotic factors (e.g. intra- and interspecific interactions, dispersal capacities and eco-evolutionary processes) (Gaston 2003, 2009; Kubish et al., 2013) have been observed to contribute to species' distribution patterns.

Environmental temperature is a major abiotic environmental driver shaping species' geographical distribution (Merriam 1894), particularly for ectotherms. In a landmark study, Janzen (1967) proposed the importance of latitudinal thermal patterns on the distribution trends for tropical species. Species with narrow thermal tolerance ranges may be favoured in the tropics due to the relatively narrow range of temperatures experienced on an annual basis. Since Janzen's time, a large body of studies has emerged looking at thermal tolerance over latitudinal gradients in several ectothermic taxa to understand physiological constraints on geographical range and predict changes in distributions associated with climate change (Addo-Bediako et al., 2000; Deutsch et al., 2008; Huey et al., 2009; Calosi et al., 2010; Sunday et al., 2011, 2012, 2014; Overgaard et al., 2014). With currently changing environmental conditions, a considerable number of range shifts have been reported in different taxa (Parmesan et al., 1999; Perry et al., 2005: Chen et al., 2011; Feary et al., 2013; Poloczanska et al., 2013; Virkkala & Lehikoinen 2014; Poloczanska et al., 2016). However, many species are unable to change their distribution range, but can adapt and evolve with change, whereas others are unable to cope (Feary et al., 2013). Still, adaptation on an eco-evolutionary basis is not always needed to cope with environmental change. Some species show high levels of plasticity in their physiological and behavioural responses to environmental variation. Understanding the factors and constraints relevant in the distribution patterns of species (Andrewartha & Birch 1954) will give us insight into basic features in biogeographical trends and allow predictions for future distribution shifts.

Intrinsic physiological traits have been suggested as a fundamental constraint on the distributions of species (Kearney et al., 2009). Due to complexity and multiple factors

involved, a macrophysiological approach is necessary to gain a full understanding of biogeographical trends in species (Somero 2005; Gaston et al., 2009; Somero 2011; Bozinovic et al., 2011; Huey et al., 2012; Naya & Bozinovic 2012; Seebacher et al., 2015). In particular, aerobic scope (AS) has been suggested to be one of the physiological limitations on geographical ranges in ectotherms (Pörtner 2001; Pörtner & Farrell 2008). AS represents the whole-animal cardio-vascular and respiratory capacity to perform simultaneous oxygen-demanding processes above those required for basic maintenance (e.g. growth, locomotion, reproduction; Fry 1971; Clark et al., 2013), and is defined as the difference between the maximum rate of aerobic metabolism (MMR) and the standard metabolic rate (SMR), referred to as the minimum of energy required to maintain all life-sustaining processes. Generally, AS in ectotherms increases with rising temperatures, is maximised at an optimum temperature and drops with further warming (Fry 1971; Farrell 2016) (see exceptions, Lefevre 2016). In theory, reductions in AS below or beyond a species' optimal temperature may cause limitations on the capacity to perform a physical activity, grow, or invest energy in reproduction (Figure 1.1). Therefore, species with a higher peak AS may encounter fewer constraints as compared to those with a lower peak AS. Species with a relatively low AS may therefore be constrained to occupy geographical regions with low variation in thermal regimes. Examining AS as a whole-animal performance trait across different species will allow us to explore physiological links and interspecific physiological differences associated with current observed distribution patterns in ectotherms.

Previous studies in fish have found positive links between AS and latitudinal distribution (Gardiner et al., 2010; Naya & Bozinovic 2012) showing that individuals or species at higher latitudinal positions or over larger distribution ranges possess higher aerobic capacities. Gardiner et al. (2010) demonstrated within several species of coral reef damselfish and cardinalfish from two source populations (Lizard and Heron Islands, 1200 km apart) that individuals found at high latitude position (Heron Islands) had higher aerobic scope performance compared to those at low latitude position (Lizard Island). Gardiner et al. (2010) results were likely due to local adaptation or plasticity via an increase in MMR for the individuals from high latitude populations. Another study on fish proposed that AS might set the upper thermal limit on the habitable regions in nine different temperate and tropical species (Payne et al., 2016), showing that a positive link between optimum temperature for AS and the highest temperature encountered in the range of teleost fish species exists. However, most of these studies focused on few species and

over relatively narrow distribution ranges (see for exception Dillon et al., 2010 (terrestrial ectotherms) and Naya & Bozinovic 2012 (aquatic ectotherms, fish)). Therefore, debate on the relevance of AS in current distribution patterns in ectotherms is continuing (Clark et al., 2013; Farrell 2013; Pörtner & Giomi 2013; Pörtner 2014; Wang et al., 2014; Farrell 2016). Our knowledge on direct links between physiological traits such AS and the observed distribution trends in diverse ectothermic taxa such fish is still unclear.

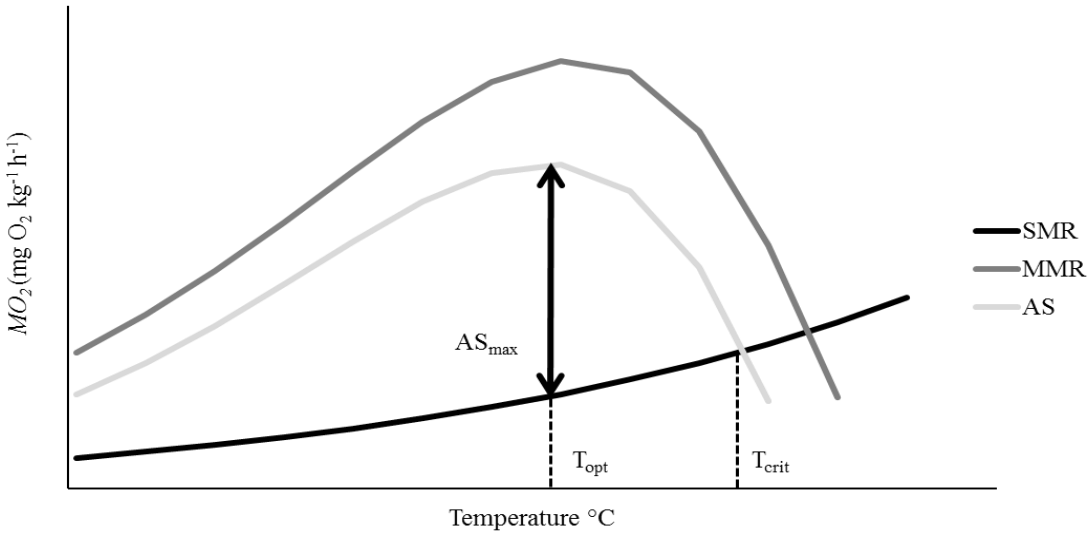



Figure 1.1 Theoretical changes in oxygen uptake ($MO_2$ mg $O_2$ $kg^{-1}$ $h^{-1}$) with temperature. SMR represents the standard metabolic rate which increases exponentially with temperature. MMR is the maximum metabolic rate, the maximum oxygen consumption during a sustainable exercise (e.g. swimming). Aerobic scope, AS, is the absolute difference between MMR-SMR, representing the whole animal cardiovascular and respiratory capacity to provide oxygen and carry out simultaneously energy demanding activities (e.g. growth, reproduction and locomotion). AS is maximised at the optimum temperature ($T_{opt}$) and start to decrease with further increasing temperatures until the critical temperature ($T_{crit}$) of the organism where AS is nil. Figure modified from Lefrançois and Claireaux 2003.

### 1.1.1 Generalist / Specialist Trade-offs

In ectotherms, different thermal responses (thermal specialist versus thermal generalist) may emerge at the species level due to eco-evolutionary adaptation towards different thermal conditions (Angilletta 2009). In theory, thermal specialists perform at a higher level but over a narrow thermal range compared to thermal generalists that are able to perform normally over a larger range of temperatures but have a reduced peak performance (Figure 1.2) (Gilchrist 1995). The presumed negative trade-off between between peak performance ($P_{max}$) at a thermal optimum and thermal performance breadth ($T_{breadth}$) arises from thermal and biochemical constraints on enzyme structure and function and membrane fluidity, which suggest that specialised adaptations for high performance at one

temperature may cause reduced performance at other temperatures. A vast literature exists over a range of taxa looking into thermal performance curves in most common measured performance traits such as locomotion (Bennett 1980), growth (Brett et al., 1969), fecundity (Berger et al., 2008) and survivorship (Selong et al., 2001). Even though theory predicts a trade-off between peak performance and performance breadth of a specific trait, most studies have not corroborated this hypothesis when examining specific traits (Huey & Hertz 1984; Purchase & Brown 2000; Ojanguren et al., 2001; Wilson et al., 2001).

Aerobic scope as whole-animal integrative performance trait has been suspected to be a major physiological constraint on species' distribution (Pörtner 2001; Pörtner & Farrell 2008). Consequently, AS responses over different thermal regimes can change and may create interspecific differences in thermal adaptation between thermal specialist and thermal generalist species. So far, performance traits examined in literature have mostly focused on isolated traits (e.g. growth, reproduction). Therefore, trade-offs related to whole organism functioning trait may fail to be detected. Additionally, these studies have focused on performance differences over different populations within a species with a few exceptions that tested the trade-off theory over a range of species. Consequently, there is still a lack of knowledge of any trade-off between breadth and peak performance exists and creates interspecific differences and constraints on thermal adaptation. AS, by virtue of being an integrative physiological trait, enables us to look into trade-offs on the whole-organism function and performance.

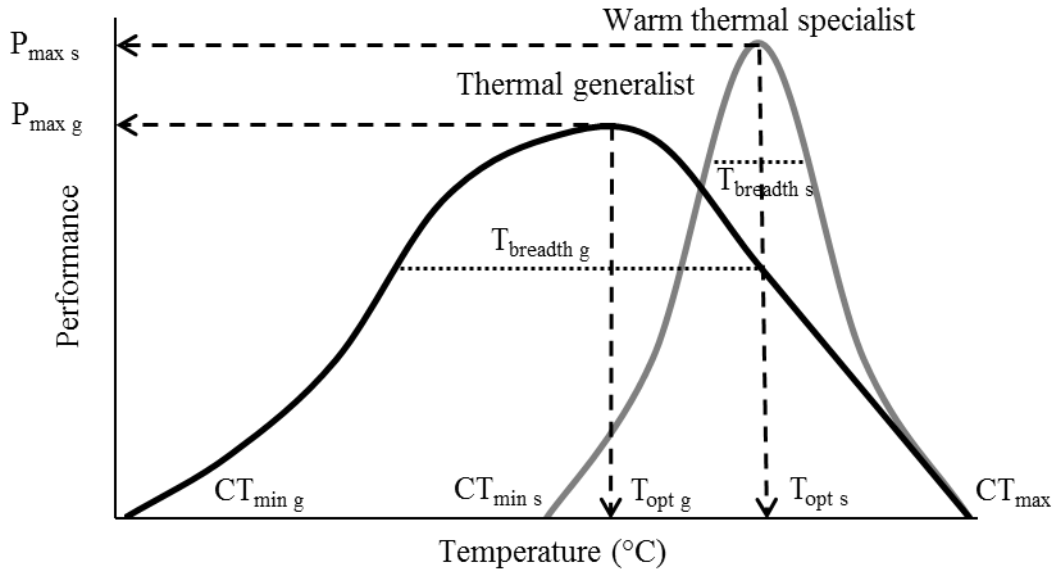



Figure 1.2 Thermal performance curve representing the concept of "A jack of all temperatures, a master of none" between thermal specialist and thermal generalist. $P_{max\ s}$ is the peak performance of thermal specialist which is higher at optimum temperature ($T_{opt\ s}$) than peak performance of thermal generalist ($P_{max\ g}$) at optimum temperature ($T_{opt\ g}$). $CT_{max}$ and $CT_{min}$ are the upper and lower thermal tolerance limits temperatures. Figure modified from Angilletta (2009).

## 1.2 Invasion ecology

In the scientific community of invasion ecologists, good colonisers or immigrants are certain specific individuals within a species or a group of species which were successful in colonising and expanding their range outside their natural native geographical boundaries (where others have failed) at a noticeable rapid range expansion rate. Human-mediated activities have however massively contributed to these colonisation events and the success of introduced individuals or species to expand and spread into the novel environments. As natural rapid range expansions do happen due to changes in environmental conditions (e.g. thermal changes or resources availability), it is crucial to distinguish between natural and human-induced range expansions. An ongoing debate between invasion ecologists still exists arguing if there is a difference between relatively recent human-aided range expansion and historic range expansion shifts occurred over the earth paleoclimatic (e.g. Brown & Sax 2005; Ricciardi 2007). It is although undeniable that human activities have reshaped and changed the geographical patterns in invasion process. As for most of the invasion biologists or ecologists, the invasion process takes place in four distinguished phases or steps (e.g. Transport, Introduction, Establishment, Spread) acting as environmental, physical or biological barriers over which individuals or species need to overcome to become a successful invader (Figure 1.3).

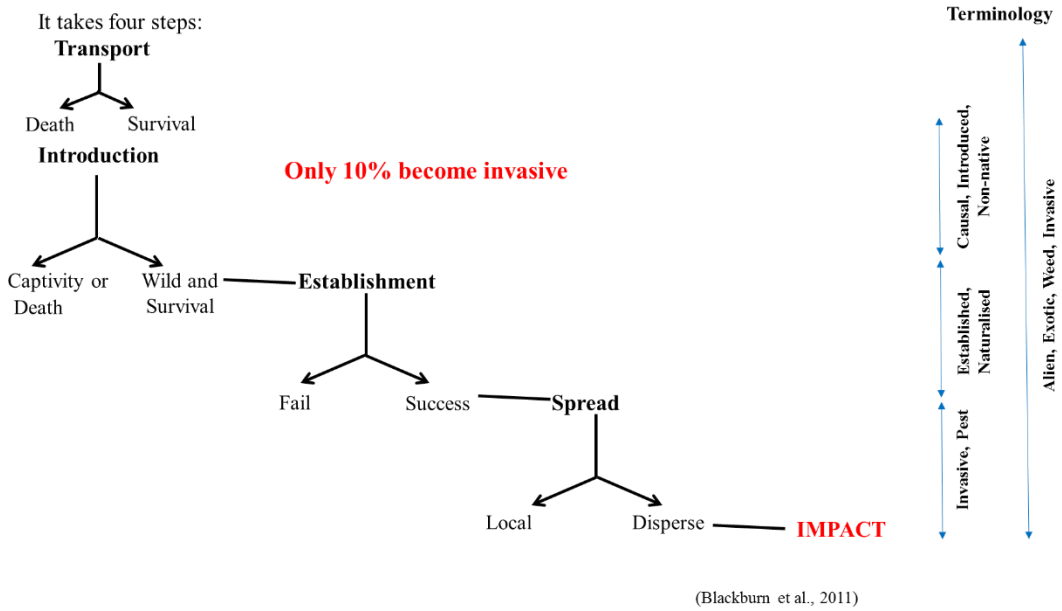



Figure 1.3 Four main proposed phases or steps in the invasion process. 1) Transport, 2) Introduction, 3) Establishment, 4) Spread and the final stage of invasion process is the negative impact invasive species might have on native species and ecosystems. Each transported and introduced individual or species needs to overcome and survive each of these different stages to be considered a successful invader. On the right hand of the figure are few examples of terminology used in the literature over the different stages of invasion process. Figure used has been modified from Figure 1 in Blackburn et al. (2011) and from Figure 1.3 from Lockwood et al. (2013).

As seen in Figure 1.3, the first stage in the invasion process is the transport phase that clearly involved direct or indirect human-mediated activities to translocate individuals or species from their native geographical range into new habitats. The common definition of a non-native species is a species which has been translocated and introduced outside of its native range by human actions. The novel introduced habitats can still be on the native continent of the introduced species, yet these new habitats could not have been colonised naturally by the species itself by expanding or shifting its distribution range. An invasive species is a species that survived and overcame all four stages in the invasion process and now is causing damages and nuisances to native ecosystems. The severity of an invasive species is classified and quantified on low to high impact scale depending on subjective human perception and perceived economic loss on damaged ecosystem services.

The introduction of invasive species into native ecosystems is a global environmental concern. Worldwide, the number of species introduced intentionally or accidentally has increased more than two-fold (Gozlan 2008) over the last 30 years (Williamson & Fitter 1996) and is still increasing due to human activities (Sala et al., 2000). Approximately 624 fish species (Gozlan 2008) have been introduced into non-native habitats worldwide which makes fishes one the most introduced taxonomic groups. In freshwater ecosystems, species

introductions are still increasing worldwide (Welcomme 1992) (Figure 1.4). The potential impacts of non-native species on ecosystems are numerous and include habitat modification (Moyle 1986; Kitchell et al., 1997), biodiversity loss due to predation and competition (Brown & Moyle 1991; Kitchell et al., 1997; McDowall 2006; Zimmerman & Vondracek 2006; Blanchet et al., 2007), introduction of disease (Gozlan et al., 2005; Gozlan et al., 2006) and hybridisation (Costedoat et al., 2004; D'Amato et al., 2007). When such impacts are widespread and destructive to native ecosystems, the introduced species is regarded as being invasive (Gozlan & Newton 2009). By causing ecological damage to native ecosystems, invasive species can also generate significant economic costs to the countries affected (for Great Britain, up to 1.7 billion pounds; Williams et al., 2010, U.S. up to 120 billion dollars per year; Pimentel et al., 2005).

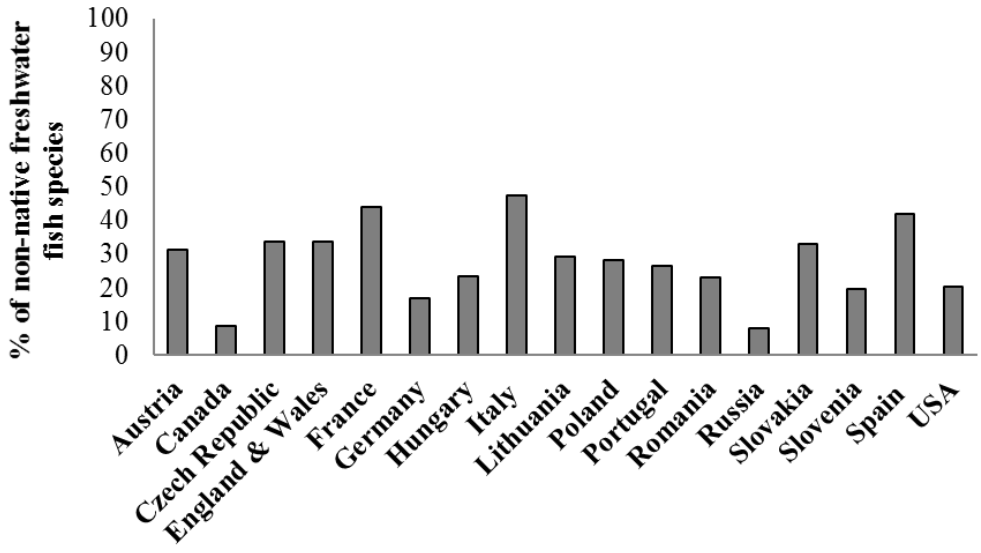



Figure 1.4 Percentage (%) of non-native freshwater fish species compared to native species per country. Data taken from Table 1 in Copp et al. (2005).

Climate change is suspected to increase the invasion rate of non-native species into aquatic ecosystems (Rahel & Olden 2008; Britton et al., 2010) by creating suitable thermal conditions for non-native warm water fish species to colonise (Sharma et al., 2007). As warmer water temperatures are expected to lead towards a shift in cyprinid and percid dominance in fish populations, changes in Northern European freshwater ecosystems are anticipated (Lehtonen 1996). In the UK, 47% of freshwater fish species are non-native. Despite the increase of introduction rate of non-native aquatic species into novel environments (Sala et al., 2000), invasion success is low (ca. 10%) and variable (Williamson 1996) (Figure 1.3). To understand and to reduce the impact of invasive

species on ecosystem functioning, it is crucial to identify the key mechanisms which contribute to the invasion success of a species. In 1958, Charles Elton, with his book *The ecology of invasions by animals and plants*, introduced a new research field "Invasion ecology". Since then a major topic in the field has been to understand how and why introduced species become successful invaders. Several life history, genetic and other biological traits have been identified to facilitate and promote invasion success (Ehrlich 1984; Moyle 1986; Sakai et al., 2001; Callaway & Ridenour 2004; Snyder & Evans 2006) (Figure 1.5). Nevertheless, specific physiological traits which contribute to invasion success are still undefined and poorly examined (Kelley 2014).

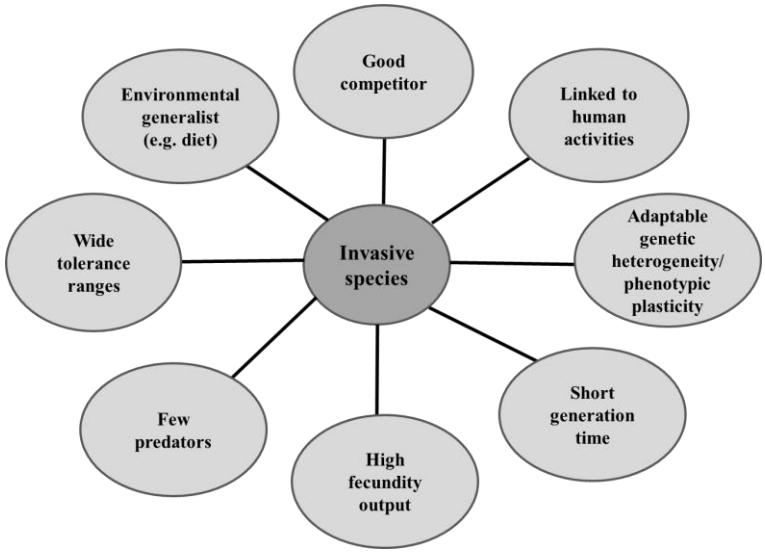



Figure 1.5 Characteristic traits involved in the invasion process and success of invasive species.

## 1.3 Physiology and invasion success

Exploring physiological attributes involved in the establishment of non-native species is an emerging research field. When introduced into novel habitats, non-native species encounter different environmental conditions (abiotic and biotic factors) compared to their native range. In order to establish, thrive and become invasive in those new environments, non-native species need to respond to those changes by adapting their physiological capacities, either from an evolutionary or plastic standpoint (Williamson & Fitter 1996; Blackburn et al., 2011). However, the particular physiological traits that permit a species to have a wide tolerance to varying environmental conditions remained undefined. A whole-animal physiological performance trait such as AS has been suggested as being relevant in invasion success (Marras et al., 2015). The SMR of a species can be a good indicator of its

capacity to cope and survive during harsh/unpredictable environmental conditions that can play a role in invasion success of a species. Having a low SMR can be beneficial and energetically less costly to maintain during severe environmental conditions whereas a higher SMR is only beneficial in certain environmental conditions (Reid et al., 2012). Comparing and examining interspecific differences in metabolic traits between native and invasive species and how they change over different environment stressors (e.g. temperatures and hypoxia) and contexts (e.g. competition) might give us an indication why, in certain circumstances, invasive species become successful and outcompete the native species.

Recently, a few studies have investigated differences in metabolic responses to thermal changes in invasive and native ectotherms. In competing amphipod species, the invasive species had a lower SMR over the range of temperatures tested (Maazouzi et al., 2011; Becker et al., 2016). In this case, the invasive amphipod may have an energetic advantage over the native species due to lower maintenance costs. Maazouzi et al. (2011) also showed that native and invasive amphipod species differ in thermal performance ranges for metabolic traits whereas invasive species was adapted to perform at colder temperatures (5-10°C) than native amphipod species (10-20°C) (Figure 1.6B). The marbled spinefoot (*Siganus rivulatus*), an invasive fish species, had a higher AS optimised at warmer temperatures (29.1°C) than native salema (*Sarpa salpa*) at 21.8°C (Marras et al., 2015) (Figure 1.6A). This would suggest that, all other things equal, invasive species with higher AS may have an advantage over native species. A higher AS could translate into higher level of flexibility in energy expenditure budget to invest in different oxygen-demanding activities such as growth, reproduction and locomotion. All these energy-demanding activities are relevant and important in the invasion process. So far, the comparative studies comparing physiological performance between invasive and native ectothermic species pairs are still rare.

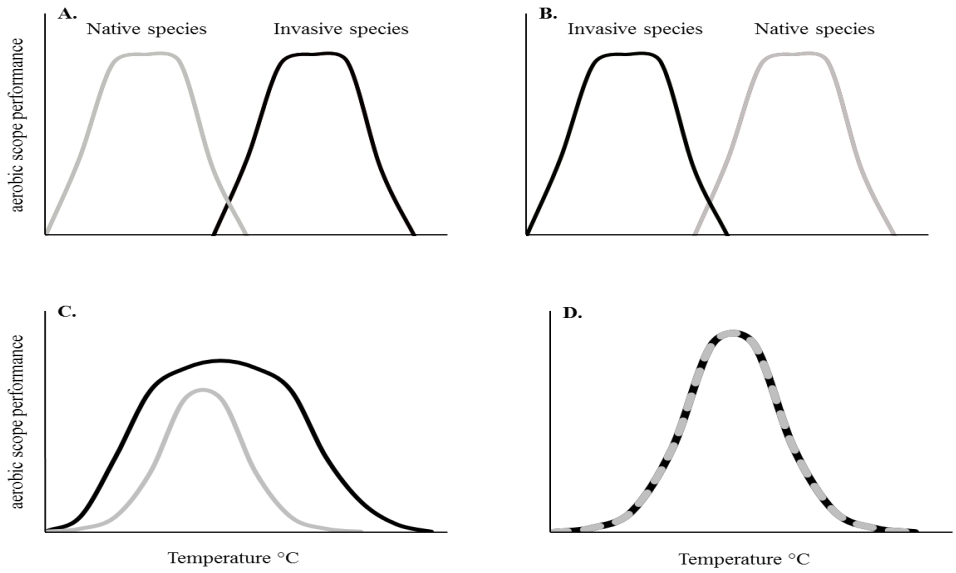



Figure 1.6 Different case scenarios of thermal performance curve for aerobic scope between invasive (black curve) and native species (grey curve). A. Invasive species perform better at warmer temperatures than native species and maximised its aerobic scope at warm temperatures (Marras et al., 2015). B. It is the opposite case, where native species perform better at warmer temperatures than invasive species (Maazouzi et al., 2011) C. Invasive species has a higher and broader aerobic scope performance curve than native over a range of temperatures. D. No differences in thermal performance curves for aerobic scope is found between invasive and native species.

## 1.4 Behavioural Traits and Invasion Success

Specific behavioural traits (such as activity level or temperature preference) might also play a significant role in the invasion success of ectothermic species. Invasion success can be linked to an ectotherm's capacity to spread and expand its distribution range. Studies of various invasive species have examined the dispersal capacities of different populations across invasion range, showing that populations at the invasion front are composed of individuals with higher dispersal capacities (Reahe & Sih 2004; Cote et al., 2010; Lopez et al., 2012; Myles-Gonzalez et al., 2015). Activity level could be related to dispersal capacity of a species. Highly active individuals tend to explore more and spread faster into novel environments (Myles-Gonzalez et al., 2015). In addition, temperature can have a profound effect on the activity of ectothermic species. With warmer temperatures, activity can increase depending on thermal sensitivity of the species (Nakayama et al., 2015), and it is therefore expected that dispersal behaviour would also increase with warmer water temperatures.

Temperature preference is another behavioural trait in species that might be relevant in the invasion process. As temperature influences habitat choice in ectotherms (Freitas et al., 2016), temperature preference might be important in the selection of the novel thermal environment an invasive species is choosing to establish and spread. In fish, temperature preference has been well studied (Coutant 1977; Jobling 1981; Kita et al., 1996; Khan & Herbert 2012; Norin et al., 2014) and can vary among different environmental conditions (van Dijk et al., 2002; Mortensen et al., 2007; Killen 2014) as well as age and reproduction stage of fish species (Jobling 1981). However, the importance of temperature preference in invasion success of species is still uninvestigated.

### 1.4.1 Competitive interactions between invasive and native species

One of the ecological consequences of the introduction of non-native species is that they can engage in competitive behaviour with native species for resources (e.g. food and/or cover availability). This interspecific competition can have major effects on species' ability to exist in a particular habitat. Several studies have focused on the competitive interactions between native and non-native fish species in habitat selection (Mills et al., 2004; Blanchet et al., 2007) and have reported negative effects on the growth efficiency and survival in native fish species (Blanchet et al., 2007; Houde et al., 2015). By competing for available resources, non-native fish species might outcompete native species resulting in possible displacement of native species into less suitable habitats.

However, the outcomes of interspecific competitions are context-dependent and can differ according to different environmental stressors mediating and changing the competitive behaviour of each species. Temperature is considered to be one of the major abiotic factors influencing interspecific competition, particularly in aquatic ecosystems (Fausch 1988; Carmona-Catot et al., 2013). The competitive outcomes will depend on the thermal sensitivity of each species regarding their physiological and behavioural traits. The variance of competitive outcomes on the prevailing environmental conditions (e.g. in response to temperature) is referred to as condition-specific competition (Taniguichi et al., 1998; McHugh & Budy 2005; Oyugi et al., 2012; Carmona-Catot et al., 2013). Condition-specific competition has been studied in range of different taxa and over different environmental stressors (Warner et al., 1993; Costanzo et al., 2005; Alcaraz et al., 2008). This condition-specific competition may be particularly relevant when it comes to invasive species (Holway et al., 2002). Thermal dependence on interspecific competition between

invasive and native species has recently attracted increasing interest (McHugh & Budy 2005; Fobert et al., 2011; Oyugi et al., 2012) and it has been shown that invasive species may be more likely to outcompete native species at warmer temperatures (Carmona-Catot et al., 2013). Indeed, the outcome of interspecific competition will influence the establishment success of non-native species into novel environments and can facilitate or diminish the invasion process.

### 1.4.2 Links between behaviour, physiology and invasion success under different environmental conditions

The performance of any behaviour may come with an energetic cost for a species, suggesting that behavioural traits (activity, temperature preference or competitive behaviour) could be linked directly or indirectly to the aerobic metabolism of species or individuals (Careau et al., 2008). A positive relationship between SMR and activity have been reported in several species, henceforth in the invasive goby (*Neogobius melanostomus*) (Myles-Gonzalez et al., 2015). In this case, species with high SMR or AS, may have the energetical benefit to perform locomotor activity but correspondingly have higher maintenance requirements due to the metabolic machinery (e.g. increased organ size and muscle mass, mitochrondrial density) necessary facilitate a higher peak level of aerobic metabolism (Killen et al., 2016). However, a negative relationship between SMR or AS and activity can also occur. In a given situation, species might face energy allocation trade-offs to maintain simultaneously multiple oxygen-demanding activities. The amount of energy attributed to maintain high SMR demands may diminish the amount of energy available to allocate to activity and vice versa. In the literature, there is evidence for both positive and negative correlations and even case studies where no links could be found between metabolic and behavioural traits over different environment conditions and ecological contexts (for details, see Metcalfe et al., 2016a).

Interspecific competitive behaviours can come with a substantial energetic cost and are likely to occupy a large portion of the AS of an animal, especially as these behaviours can involve intense physical activity (Smith & Taylor 1993; Huntingford et al., 1995; Hack 1997; Briffa & Sneddon 2007). Studies have found that individuals with higher SMR tend to be more aggressive and dominant (Metcalfe et al., 1995; Cutts et al., 1998). So far, most studies have examined links between metabolic traits and competitive behaviour on intraspecific differences between SMR and dominance in fish (Metcalfe et al., 2016a).

Only few studies have examined the relationship between AS and competitive behaviour in fish. Killen et al. (2014) found a positive correlation between AS and aggressiveness in juvenile damselfish during intraspecific competition for territory. Seth et al. (2013) also showed a positive link between AS and interspecific competition for shelter in sculpin species from different distribution ranges. Species or individuals with an increased AS have the capacity to engage in competitive behaviours and win the interactions while still be able to carry out other physiological tasks such as growth or digestion. Although several studies have investigated differences in behavioural and performance traits between native and invasive species (aggression and habitat selection Mills et al., 2004; for habitat use/selection Blanchet et al., 2007; Kakareko et al., 2014) over thermal gradient (Fobert et al., 2011; Oyugi et al., 2012; Carmona-Catot et al., 2013), none have examined how differences in competitive behaviours may be related to AS. Examining links between physiological traits, such as AS, and competitive behaviours would be useful for understanding a variety of ecological phenomena including competitive interactions between native and invasive species during range expansions. All associations between behavioural traits and physiology may change depending on environmental context (e.g. temperature and hypoxia) (Killen et al., 2013). According to tolerance range and plasticity in physiological and behavioural responses towards the environmental factor examined, one species might have an advantage under one set of conditions while the other species gains advantage once conditions change. A successful invasive species is likely to have wider tolerance ranges towards environmental variability; it is therefore likely that invasive species will have the required physiological and behavioural traits to outcompete native species over a range of conditions.

## 1.5 Study Species

The two study species used in this research project are both benthic bottom dwelling species, bullhead (*Cottus gobio*, Linnaeus 1758) and stone loach (*Barbatula barbatula*, Linnaeus 1758) (Figure 1.7).

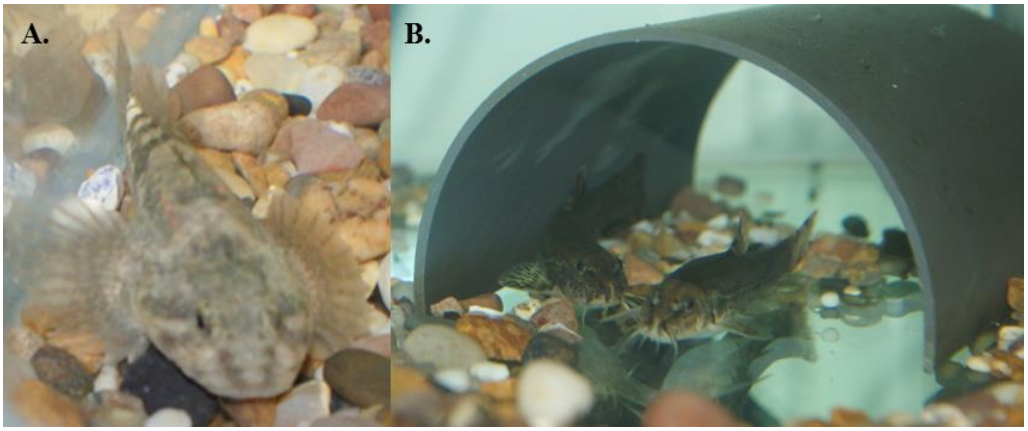


Figure 1.7 A. Invasive bullhead and B. Native stone loach.

### 1.5.1 Distribution range

Bullheads can be found all over Europe, ranging from Greenland and Scandinavia to Italy (Regan 1911; Smyly 1957) and up to around 2000 m above sea level (Fatio 1882). In Britain, it is the only freshwater species of the Cottidae family and is commonly found in England and Wales. In Scotland, bullheads have been introduced in Clyde River catchments (around Glasgow) and streams around Edinburgh by anglers who used them as live bait for pike fishing. Since then, bullheads have established self-sustaining population in the Clyde River catchment (Maitland & Campbell 1992; Yeomans & Jennings 2013) and are expanding their distribution range (Yeomans W. pers. comments; Yeomans & Jennings 2013). Bullheads are now considered highly invasive in Scotland (Figure 1.8). They are mostly found in stony streams and rivers with weak to moderate flow regimes and high oxygen content (Symly 1957; Tomlinson & Perrow 2003).

Stone loaches are widely distributed in Europe as well and in Asia. In Britain, they can be found almost everywhere except in the north of Scotland (Figure 1.8). Stone loaches occur in still and flowing rivers at sea level to up 300 m above sea levels. They are found in

waters under the ice up to rivers at 21°C (Symly 1955). Stone loaches are native in Scottish rivers.


Figure 1.8 Distribution range of (A) bullhead and (B) stone loach in the British Islands. The grey circles represent the location from which I collected the fish in this project. Maps have been extracted from (http://data.nbn.org.uk).

### 1.5.2 Behaviour

Bullheads are known to have a sedentary and solitary lifestyle, mainly hiding under rocks and stones (Symly 1957). As they have a high predation risk, their preferred habitats have an abundance of shade and cover. Adult bullheads are territorial, defending their shelter from competitors by using sound or visual threat displays and sometimes aggressive fighting. Intraspecific competition for shelter is size-related; larger individuals chase small individuals away from the shelter. Symly (1957) and Pecl (1990) have observed homing behaviour towards a permanent shelter in bullheads; they can stay and turn back to the same stone for years reflecting high site fidelity. Due to reduced swimming ability, their primarily locomotion features are hopping movements using their pectoral fins and occasionally performing burst swims. Depending on population density and seasonal events (e.g. spawning season) (Knaepkens et al., 2004), bullheads have been recorded to move on average 4-10 m to 12-48 m (Downhower et al., 1990). The foraging ambush behaviour of bullheads reflects their sedentary lifestyle. They mainly feed at dusk/dawn or during the night (Welton et al., 1991) and are relatively inactive during the day. Their main diet consists of benthic invertebrates but may vary according to seasons and availability of

food items. Bullheads seem to be an opportunistic predator and prefer to select their protective habitat (under shelter) than any particular diet (Welton et al., 1991).

Stone loaches are also known to be poor swimmers even though sustained swimming has been observed (Tudorache et al., 2008) which can be an adaptation to the environment that they inhabit (fast streaming rivers, creeks or streams), occasionally performing small migrations of up to 100 m (Tudorache et al., 2008). They are active foragers, searching for food in the substrate by using their sensory tactile barbels (Smyly 1955). Like bullheads, they mainly forage during night time and are relatively inactive during the day (Symly 1955), mainly to avoid predation. Stone loaches are more gregarious than bullheads and several individuals of stone loaches were even found together with other fish species (e.g. minnows) under the same shelter. The main food items of stone loaches are benthic invertebrates (Symly 1955).

### 1.5.3 Thermal tolerance

Bullheads and stone loaches differ in their upper thermal limits ($CT_{max}$) depending on age and acclimation temperatures. Adult bullheads have lower $CT_{max}$ (27.6°C) than stone loaches (29.1°C). The lower thermal limits ($CT_{min}$) are the same in both species, 0-3°C for stone loaches and for bullheads 0-2.5°C. Feeding ceases in both fish species for bullheads at 26.5°C and stone loaches at 28°C (Elliott et al., 1994, 1995). The optimal growth temperature for stone loaches is 19°C (Elliott et al., 1996) whereas bullheads grow better at colder temperature (~10°C, 14°C), depending on age and thermal history (Abdoli et al., 2007).

### 1.5.4 Hypoxia tolerance

Both bullheads and stone loaches require richly oxygenated waters. Stone loaches are able to store oxygen in the intestines and possible use them as a supplementary breathing organ (Maitland 2007). However, hypoxia tolerance limits are not defined in either benthic species.

### 1.5.5 Interactions

Stone loaches and bullheads are known to co-exist over their native range (Wheeler 1969) sharing the same ecological niche. They compete for resources such as shelter (Prenda et al., 1997a). While competing for food items, these species seem to co-exist by habitat

partitioning (Welton et al., 1983; Welton et al., 1991). Nevertheless, the degree of co-existence may be dependent on the population density of each species. Symly (1957) observed a negative correlation for presence/absence ratio between these two fish species and a mutual avoidance behaviour. In rivers where bullheads were highly abundant, stone loaches were rarely found (Yeomans W. pers. comments). The opposite was true as well, when population density of bullheads decreases, an increase of stone loaches was observed (Mann 1989).

## 1.6 Overall aims

The main aim of this PhD project is to contribute to the understanding of how the whole-animal metabolic traits and behaviour determine distribution patterns, thermal adaptation and invasiveness in fish. I address these questions by a combination of broad comparative analyses and more specific experiments on native stone loaches (*Barbatula barbatula)* and invasive bullheads (*Cottus gobio*) in Scotland. Investigating the underlying mechanisms which lead to the invasion success of non-native fish species and the decline of native fish species is crucial for predicting future changes in fish populations and communities. The exact mechanisms which allow a non-native species to become invasive and spread are still relatively poorly understood. I focus on examining differences in AS and behavioural traits between native and invasive freshwater fish species over different environmental conditions (e.g. different temperatures and hypoxia). The series of comparative studies and experiments performed over this PhD project will give a better insight and understanding of the possible role played by the aerobic metabolism in explaining the observed biogeographical distribution trends, thermal adaptation and invasion success in fishes.

### 1.6.1 Chapter 2: The effect of metabolic traits on the geographic distribution and invasion success of teleost fishes

In this chapter, the main objective was to examine the links between AS and observed latitude distribution and invasion success in fish. Therefore, I collected AS data from the literature and conducted comparative analyses to answer two key questions; 1) Is aerobic performance linked to distribution range in fish? and 2) Is invasiveness success determined by aerobic performance? The importance of physiological traits such as aerobic capacity in determining the latitude range and invasion success in ectotherms such as fish is still poorly understood. I had two main hypotheses in this study, 1) Fish with higher AS would

have a wider distribution range compared to fish with lower AS and 2) Invasive fish species might have a higher AS than native species which could explain on energetic basis why invasive species are better competitors.

### 1.6.2 Chapter 3: Is there a trade-off between peak performance and performance breadth across temperatures for aerobic scope in teleost fishes?

In this chapter, I was interested in testing the potential trade-off between being a thermal specialist or thermal generalist. This hypothesis suggests a negative correlation between peak and performance breadth for a specific trait across thermal range. I focused on AS as a performance trait and conducted a phylogenetically-informed comparative analysis of the relationship between performance peak and breadth for AS across teleost fishes.

### 1.6.3 Chapter 4: Metabolic and behavioural responses to thermal variation in an invasive and native freshwater fish species

The aim of this chapter was to compare physiological and behavioural responses of a native fish species, stone loach (*Barbatula barbatula*), and an invasive fish species, bullhead (*Cottus gobio*), across their current thermal range (summer temperatures: 13-21°C) in order to examine the invasion success of bullheads in Scottish rivers. Bullheads have been successful in colonising and are currently spreading in their introduced habitats in Clyde River catchments (Yeomans & Jennings 2013). These pairs of species were chosen for the following experimental chapters (Chapters 4, 5 and 6) for several reasons; 1) high densities in invasive bullheads have been observed in Clyde River catchments, 2) both species seemed to display mutual avoidance behaviour and finally resulting from this 3) it seemed that native stone loaches populations have been affected and displayed a reduction in abundance possible due to high numbers of bullheads present. It is still unclear what characteristics or traits (physiological and/or behavioural) have contributed to invasion success of bullheads in Scottish rivers, how they compared to native stone loaches and how they might change under different environmental conditions (e.g. in this chapter, under different thermal regimes). To determine whole-animal physiological performance traits such as metabolic rates in all my experimental chapters (Chapters 4, 5 and 6), I used indirect calorimetry methods, intermittent-closed respirometry technics. In this chapter, I quantify and compared metabolic traits (SMR, MMR and AS) in both species during acute

and acclimated thermal trials and behavioural traits (activity levels and temperature preference). Activity levels were tested in this chapter to get an estimate of the dispersal potential to spread into a novel environment for each fish species. As for the thermal preference trials, I was interested to observe which thermal habitats they might prefer to spread into given a choice. A second objective in this chapter was to test for associations between physiological and behavioural traits within both species. The main hypothesis in this chapter was to observe differences in metabolic and behavioural traits between bullheads and stone loaches. I expected bullheads to have a different thermal metabolic phenotype and to possess a higher AS over all tested temperature range than stone loaches. These expected differences might have favoured the invasion success of bullheads in Scottish rivers.

### 1.6.4 Chapter 5: The effect of thermal tolerance on competitive outcomes between an invasive and a native freshwater fish species

Both of my study species can co-exist in the same river systems, therefore they can potentially compete for the same resources (e.g. food and/or cover availability). In this chapter, I compared the competitive ability between bullheads (*Cottus gobio*) and stone loaches (*Barbatula barbatula*), using a single shelter as the target resource at three different acclimation temperatures (13°C, 17°C and 21°C). Metabolic rate data (SMR, MMR and AS) were collected as well at all temperatures for individual fish involved in competition trials to investigate the possible association between intra- and interspecific competitive ability and metabolic traits. The main questions in this chapter were; 1) Is competitive ability between bullheads and stone loaches linked to interspecific differences in aerobic scope? 2) Is competitive ability related to aerobic scope within species?; and 3) Does temperature modulate competitive ability or links between competitive ability and aerobic scope? My main hypothesis for this chapter was that the invasive bullhead might have higher competitive capacity for shelter over all temperatures tested, and that this might be linked to higher aerobic scope capacity compared to stone loaches.

### 1.6.5 Chapter 6: Physiological and behavioural responses to hypoxia in invasive and native freshwater fish species

One of the characteristics defining a good invader is the capacity to cope and thrive in disturbed environments. In unfavourable conditions, such as hypoxic episodes, invasive species might be better adapted and flexible in their physiological and behavioural

responses towards this stressor. In this chapter, I compared physiological (e.g. metabolic rate, $P_{crit}$ value) and behavioural traits (avoidance behaviour with a shelter opportunity provided in hypoxic conditions) between bullheads (*Cottus gobio;* invasive) and stone loaches (*Barbatula barbatula*) over different dissolved oxygen (DO) concentrations in the water (100%, 80%, 60%, 40%, 30%, 25% and 20% DO levels) with the aim to look at the coping capacities between these species in hypoxic conditions. The two main questions in this chapter were: 1) Are bullheads more hypoxia tolerant (i.e. have a lower $P_{crit}$) than stone loaches? and 2) Do bullheads and stone loaches differ in their hypoxia avoidance behaviour? The main hypothesis was that bullheads might have a higher hypoxia tolerance adaptation than native stone loaches.

# Chapter 2: The effect of metabolic traits on the geographic distribution and invasion success of teleost fishes

Collaboration work with: Paul Johnson, Enrico E. Rezende, Lewis Halsey. Currently in preparation to be submitted to Physiological and Biochemical Zoology

## 2.1 Abstract

Abiotic as well as biotic factors are relevant in shaping species' distribution and invasiveness but our knowledge is still lacking on the importance of physiological traits such as aerobic capacity in determining the latitude range and invasion process in ectotherms such as fish. Here, I collected metabolic rate data (e.g. aerobic scope, AS) from literature to address two key questions; 1) Is aerobic performance linked to distribution range in fish? and 2) Is invasion success affected by aerobic performance? I found no evidence for the direct implication of peak aerobic scope performance as a whole-animal performance trait in the present observed latitudinal trends for 86 fish species. As for invasiveness in 59 freshwater fish species (23 invasive and 36 native species), aerobic scope did not explain invasion success in fish. The results here suggest that direct effect of AS may be less important in constraining the distribution of native and invasive teleost fish species than it has previously been speculated.

## 2.2 Introduction

The geographical distributions of species are shaped by several interacting abiotic (e.g. temperature and humidity) and biotic factors (e.g. intra- and interspecific interactions and dispersal capacity) (Gaston 2003, 2009; Kubish et al., 2013). In response to changing environmental conditions, range shifts have been observed in numerous taxa (Parmesan et al., 1999; Perry et al., 2005; Chen et al., 2011; Feary et al., 2013; Virkkala & Lehikoinen 2014). Although many species are unable to shift their distribution range, they can adapt and evolve with change, whereas others are unable to cope and fail in changing distribution limits (Feary et al., 2013). Still other species might not need to show evolutionary adaptation to changing conditions due to high plasticity in their physiological and behavioural responses to environmental change. Knowing and understanding the relevant factors and constraints influencing the distribution patterns of species (Andrewartha & Birch 1954) will provide insight of the basic features in biogeographical trends and allow predictions for future distribution shifts.

Intrinsic physiological traits also provide a fundamental constraint on the distributions of species (Kearney et al., 2009). Indeed, it has been suggested that a macrophysiological approach is necessary to achieve a full understanding of biogeographical trends in species (Somero 2005; Gaston et al., 2009; Somero 2011; Bozinovic et al., 2011; Huey et al 2012; Naya & Bozinovic 2012; Seebacher et al., 2015). For ectotherms in particular, aerobic scope has been proposed to be one of physiological limitations on geographical ranges (Pörtner 2001; Pörtner & Farrell 2008). Aerobic scope represents the cardio-vascular and respiratory capacity to perform simultaneous oxygen-demanding processes above those required for basic maintenance (e.g. growth, locomotion and reproduction; Fry 1971; Clark et al., 2013). Aerobic scope in ectotherms often increases with rising temperature, peaks at an optimum temperature and drops with further warming (Fry 1971; Farrell 2016) (see exceptions, Lefevre 2016). In theory, decreases in aerobic scope below or beyond a species' optimal temperature may impose constraints on the ability to perform physical activity, growth, or invest energy in reproduction. A species with a higher peak aerobic scope may be less constrained as compared to those with a lower peak. Consequently, species with a higher peak for AS may have the aerobic capacity to cope or endure a high thermal variability and still be able to allocate sufficient energy to different activities. In contrast, species with a relatively low aerobic scope may only be able to occupy geographical regions where thermal conditions are stable and do not vary significantly.

Examining aerobic scope as a whole animal performance trait across different species will allow us to explore physiological links and interspecific physiological differences associated to current observed distribution patterns in ectotherms.

Several studies in endotherms have shown positive links between whole-animal metabolic traits and geographic distributions (basal metabolic rate, BMR Lovegrove 2000; maximum and basal metabolic rate, MMR and BMR Rezende et al., 2001, 2004; aerobic scope AS Naya et al., 2012.). As for example, a positive correlation between aerobic scope and latitude was found across 48 rodent species (Naya et al., 2012). In ectotherms, most studies examining metabolic traits (SMR and AS) linked to distribution trends so far have focused on few species over a relatively narrow latitudinal gradient (Gardiner et al., 2010, Whiteley et al., 2011; Rummer et al., 2013; Payne et al., 2016). Gardiner et al. (2010) showed that on intraspecific level, among two source populations (Heron and Lizard Island) of coral reef damselfish and cardinalfish populations within species at a high latitude (Heron Island) had a higher AS compared to the fish populations of the same given species occurring at low latitude (Lizard Island). Over a broader geographical scale and interspecific level Naya & Bozinovic (2012), found as well a positive correlation between latitude range and metabolic scope in 38 fish species, though they did not account for phylogeny relatedness between the different fish species.

A better understanding of the physiological traits that influence distribution ranges will also provide insight into the factors that may allow non-native species to colonise new habitats and potentially become invasive. Invasive species can produce major disruptions to native ecosystems and there is an ongoing research effort to understand the life history, behavioural and genetic traits that allow certain species to become successful invaders (Ehrlich 1984; Moyle 1986; Williamson & Fitter 1996; Bates et al., 2013; Kelley 2014). In contrast, however, the role of physiological traits in invasion success has been largely overlooked and to date remains poorly understood (Cooke et al., 2013; Bates et al., 2013; Kelley 2014; Lennox et al., 2015). A few studies have found differences in metabolic traits (SMR and AS) between native and invasive ectothermic species (Maazouzi et al., 2011; Tomlinson et al., 2015; Marras et al., 2015; Becker et al., 2016). Lower maintenance costs of living (SMR) were found in invasive species compared to the native gammarid species (Maazouzi et al., 2011; Becker et al., 2016). Possessing low standard metabolic rates (SMR) can be beneficial to survive and cope during harsh and/or unpredicted conditions (Reid et al., 2012). However, aerobic scope may also be relevant for determining the

invasiveness of species, potentially limiting locomotion, growth or reproduction during the different stages of invasion (transport, introduction, establishment and spread). All else being equal, an invasive species with a relatively high aerobic scope may have an energetic advantage, allowing it to outcompete and displace native species, especially if thermal regimes shift closer to the thermal optima of the invasive species (Marras et al., 2015).

In this study, I used a comparative approach to examine links between aerobic scope and geographical distribution across 86 species teleost fishes. Furthermore, using a subset of this data, I sought to determine whether aerobic scope plays a role in determining which species become invasive. Teleost fish are a diverse taxon found in all aquatic habitats covering a large and diverse geographical range. Furthermore, the last several decades have seen an extensive research effort into quantifying metabolic traits in different fish species. Fishes are also the most introduced aquatic taxa worldwide (Welcomme 1998) and several invasive fish species have caused ecosystem disruptions in habitats across the globe. Specifically, I was interested in addressing two main questions: 1) Is aerobic performance linked to absolute latitude distribution range in fish?; and 2) Is invasion success linked to aerobic performance? The results here provide insight into the physiological factors affecting the present-day distributions as well as how changes in temperature may alter species' ranges and potentially facilitate the spread of invasive species into non-native habitats.

## 2.3 Methods and Materials

### 2.3.1 Data collection

(a) Metabolic rate data

Literature data on the standard and maximum metabolic rates of 92 species of fish were collected from the literature. Standard metabolic rate (SMR; mg $O_2$ $h^{-1}$) is defined as the minimum energy required to sustain life and maximum metabolic rate (MMR; mg $O_2$ $h^{-1}$) is defined as the maximum rate of aerobic metabolism that is able to be performed by an animal. Whole animal absolute aerobic scope (AS; mg $O_2$ $h^{-1}$) was calculated as the difference between MMR and SMR. To obtain literature data, I searched Web of Knowledge and Google Scholar using keywords "standard metabolic rate" or "maximum metabolic rate" or "aerobic scope" and "fish"). Species were only included when both SMR and MMR were measured in the same study. For SMR, I only considered studies

which measured metabolic rates as followed 1) by extrapolating oxygen consumption values measured at different activity levels to zero activity level or 2) by direct recording of oxygen rates of a post larval, starved and resting fish over a consistent period of time; For MMR 1) by recording critical peak oxygen rates during forced swimming in swim tunnel, or 2) by following up immediately after exhausting exercise in swim tunnel or 3) after a chasing protocol. Besides collecting $MO_2$ data from each study, I also recorded the mean body mass (g) of the fish and acclimation temperature used in each study. In cases where metabolic rates ($MO_2$) were tested over more than one temperature, I used the data for AS at the temperature at which AS was maximised to obtain the peak performance for AS.

(b) Latitudinal and life history data

Latitudinal range data were obtained from FishBase (Froese & Pauly 2014) for 87 fish of the 92 species for which metabolic rate data was available. I also collected information from FishBase on species' lifestyle (benthic, benthopelagic and pelagic), habitat (marine, freshwater and marine/freshwater) and maximum length (cm). Absolute latitude range of each species was used in this analysis as an indicator of current distribution range, corresponding to a range from 0-90°. For any species having a distribution range overlapping the equator (e.g. 64°N - 18°S), I considered the absolute latitude range expanding from equator up to maximum latitudinal point of the species in the Northern Hemisphere (here in this case 64°). The reasons for which I acknowledged to take the distribution range expanding to the Northern Hemisphere to calculate the absolute latitude range for each species were: 1) most $MO_2$ data taken in this study are on fish species with a Northern hemisphere distribution (n = 59) and 2) there are notable differences in the sea temperature variation between Northern and Southern Hemisphere, with higher thermal variability in the Northern Hemisphere (Sunday et al., 2011, see Figure 1b.). While a few species distribution range were overlapping with equator (n = 27), I took their Northern Hemisphere distribution range to be consistent in my dataset regarding the thermal variation each one of the species in the dataset is potentially experiencing. Latitudinal midpoint position for each of 87 fish species was calculated from the average of the distribution range (considering Northern Hemisphere values as positive and Southern Hemisphere as negative values) for each species and categorised into three categories according to species' latitudinal midpoint position, tropical (0-20°), temperate (20-60°) and polar (> 60°) (Figure 2.1).

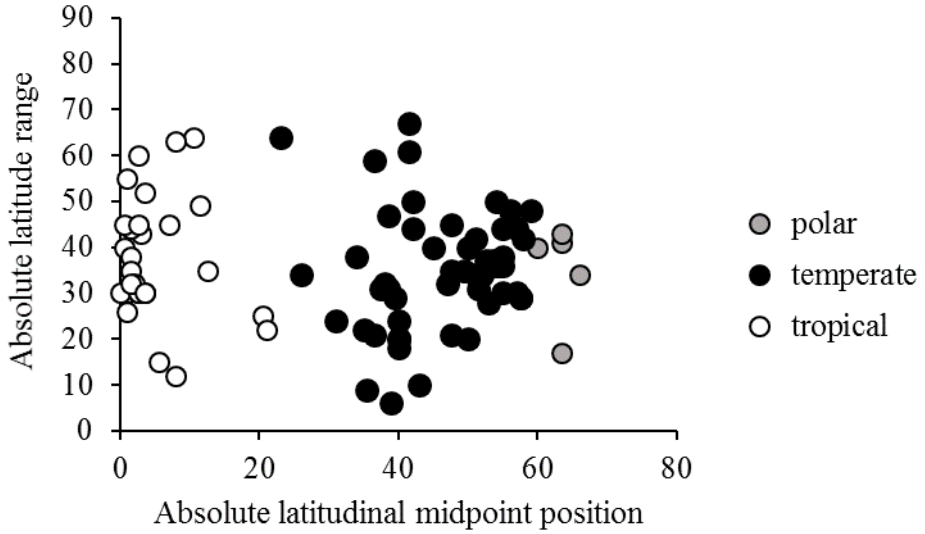



Figure 2.1 Relationship between absolute latitude range and absolute latitude midpoint position in 86 fish species categorised into tropical (white dots, n = 29), temperate (black dots, n = 52) and polar (grey dots, n = 5) species.

(c) Invasiveness and life history data

For the invasiveness model, I concentrated only on freshwater fish species for which most of metabolic rate studies were found (59 species in total, 23 invasive and 36 native species). For marine fish species, I found $MO_2$ data for only 1 invasive species (*Siganus rivulatus*, Marras et al., 2015). I considered the status of invasive species by taking as a reference FishBase coding invasive species as "potential pest". Additionally, I focused on the fish order Cypriniformes, for which most of invasive species were found in this dataset (n = 27 species, 11 invasive versus 16 native) and this order includes most of the known invasive freshwater fish species up to date. As for the latitudinal dataset, I extracted information on lifestyle (benthic, benthopelagic and pelagic) and maximum length (cm) from FishBase alongside with metabolic rate data.

### 2.3.2 Data analysis

(a) Absolute latitude range model

To account for differences in phylogenetic relatedness among the 86 fish species (the only Southern Hemisphere species in the dataset, *Pagothenia borchgrevinki*, was not included), a phylogenetic tree was constructed using primarily phylogenetic information by Near et

al. (2013) on spiny-rayed fishes (Acanthopmorphs) and by Wang et al. (2012) and Yang et al. (2015) on carps (Cypriniformes) (Figure 2.2). The common ancestor of the resulting phylogenetic tree was set at around 275 mya (based on two trees obtained by (Wang et al., 2012 and Near et al., 2013)). Additional information on Elopomorpha (eels) was obtained from Betancourt et al. (2013). Congeneric species were included in monophyletic groups within polytomy in order to increase resolution (for *Oncorhynchus, Salvelinus, Gadus* and *Myoxocephalus*). Unresolved nodes were maintained as soft polytomies and the node depth was set to half of the total depth in these cases.

The statistical model on the absolute latitudinal range in fish was analysed using phylogenetic generalised least squares method (PGLS, Grafen 1989; Garland & Ives 2000) with caper package (Orme 2013) in RStudio (version 3.3.0 R Foundation for Statistical Computing), applying a phylogeny generated from the comprehensive tree of life (Figure 2.2) (Hinchliff et al., 2015) using the 'rotl' package (Michonneau et al., 2015). A measure of phylogenetic correlation, λ (Freckleton et al., 2002) was estimated by fitting PGLS models with different values of λ and finding the value that maximises the log likelihood. Thus, the degree to which trait evolution deviates from Brownian motion (λ = 1) was determined by modifying the covariance matrix using the maximum-likelihood value of λ, which is a multiplier of the off-diagonal elements of the covariance matrix (i.e. those quantifying the degree of relatedness between species). To test the relationship between distribution range and aerobic scope in fish, the following PGLS model was used:

$$\text{Absolute latitude range} \sim \alpha_0 + \alpha_1 \log \text{AS} + \alpha_2 \log \text{mass} + \alpha_3 \text{temperature} + \alpha_4 \text{latitudinal midpoint position} + \varepsilon$$

where absolute latitude range represented species' absolute latitude range from 0-90°, log AS was log-transformed aerobic scope (mg $O_2$ $h^{-1}$), log mass was log-transformed mass (g) of each species used, temperature was the test temperature (°C) at which aerobic scope was measured in each study, latitudinal midpoint position was the categorised midpoint position of each fish species (tropical, temperate and polar) and ε represented the unexplained variation in the dataset. Mass and acclimation temperature were included in the model for several reasons; due to 1) different scaling effects on aerobic scope and to correct for these effects on AS, 2) account for range and differences in mass and temperature tested over various species in the dataset and 3) consider the independent effects of those two covariates on absolute latitude range of species. Aerobic scope and

mass were log-transformed in the model due to allometric relationship between those two covariates. Latitudinal midpoint position was included in the model to adjust and consider thermal stability and variability across latitude. Daily, seasonal and annual temperature variations vary in magnitude depending on latitudinal position, with high thermal fluctuations at temperate latitudes and low thermal variations at tropical and polar latitudes (Sunday et al., 2011). Thermal history experienced by a species affecting all physiological processes depends on latitudinal midpoint position. Therefore, latitudinal location of species can have implication on its aerobic performance.

(b) Invasiveness models

In contrast to the model above, to test the link between invasiveness and aerobic capacity in 59 freshwater fish species, I used a binomial response mixed-effect model instead of a phylogenetic generalised least squares method (PGLS) as the response variable is binomial (invasive = 1, native = 0). The PGLS approach cannot been used on binomial data. To control for phylogenetic dependency, I included order (n = 14) as a random effect in the model. I fitted the following mixed-effect binomial model:

$$\text{Invasiveness} \sim \alpha_0 + \alpha_1 \log \text{AS} + \alpha_2 \log \text{mass} + \alpha_3 \text{temperature} + \alpha_4 \log \text{length} + (1|\text{order}) + \varepsilon$$

where Invasiveness was a binomial response variable (invasive = 1, native = 0), log AS was log-transformed aerobic scope (mg $O_2$ $h^{-1}$), log mass was log-transformed mass (g) of each species used, temperature was the test temperature (°C) at which aerobic scope was measured in each study, log-transformed maximum length (cm) for each species extracted from FishBase and ε represented the unexplained variation in the dataset. Mass and acclimation temperature were included in this model for the same reasons as mentioned in absolute latitudinal range model. Maximum length was integrated in the model as a proxy for fecundity as larger fish tend to produce more eggs (Elgar 1990). In terms of invasiveness, the number and quality of offspring released into a novel environment referred to as propagule pressure, is relevant and important for the establishment progress and success of non-native species and can play role in geographical spread and dispersal of a species.

Within Cypriniformes order (n = 27 species), I used the same binomial model as described above but without the random effect included in the model structure.
All statistical analyses were performed in RStudio v.3.3.0 using caper package (Orme, 2013) for absolute latitudinal PGLS model and lmerTest package (Kuznetsova et al., 2016) for invasiveness models. The significance level of all tests was p = 0.05.

## 2.4 Results

(a) Absolute latitude range model

PGLS analysis did not reveal any relationship between absolute latitude range and peak aerobic performance (PGLS, effect of log AS, t = -1.344, p = 0.183; Table 2.1; Figure 2.3). Geographical location (tropical, temperate and polar) did not explain absolute latitude range in fish (for details see Table 2.1; Figure 2.3). The relationship between absolute latitude range and aerobic scope was independent of phylogenetic relatedness between species (Table 2.1, $\lambda = 0.00$, Figure 2.2). PGLS model here in this study explained 17.9 % of observed variation in absolute latitude range in fish.

Table 2.1 Summary of the PGLS model testing for the effects of aerobic scope (log AS (mg $O_2$ $h^{-1}$)), mass (log g), tested temperature (°C) and absolute midpoint position (tropical, temperate and polar) on absolute latitude range (0-90°). $R^2 = 0.179$, $F_{5, 80} = 3.484$, p = 0.007, n = 86 species, $\lambda = 0.00$. For latitude midpoint categorisation, the reference category is 'tropical'.

| **term** | **estimate** | **s.e.m** | ***t*** | ***p*** |
|---|---|---|---|---|
| Intercept | 28.209 | 8.903 | 3.168 | 0.002 |
| logAS | -5.82 | 4.331 | -1.344 | 0.183 |
| logmass | 9.965 | 4.316 | 2.309 | 0.024 |
| temperature | 0.075 | 0.283 | 0.265 | 0.792 |
| **Midpoint** | | | | |
| temperate species | -5.996 | 3.51 | -1.708 | 0.091 |
| polar species | -7.769 | 7.056 | -1.101 | 0.274 |

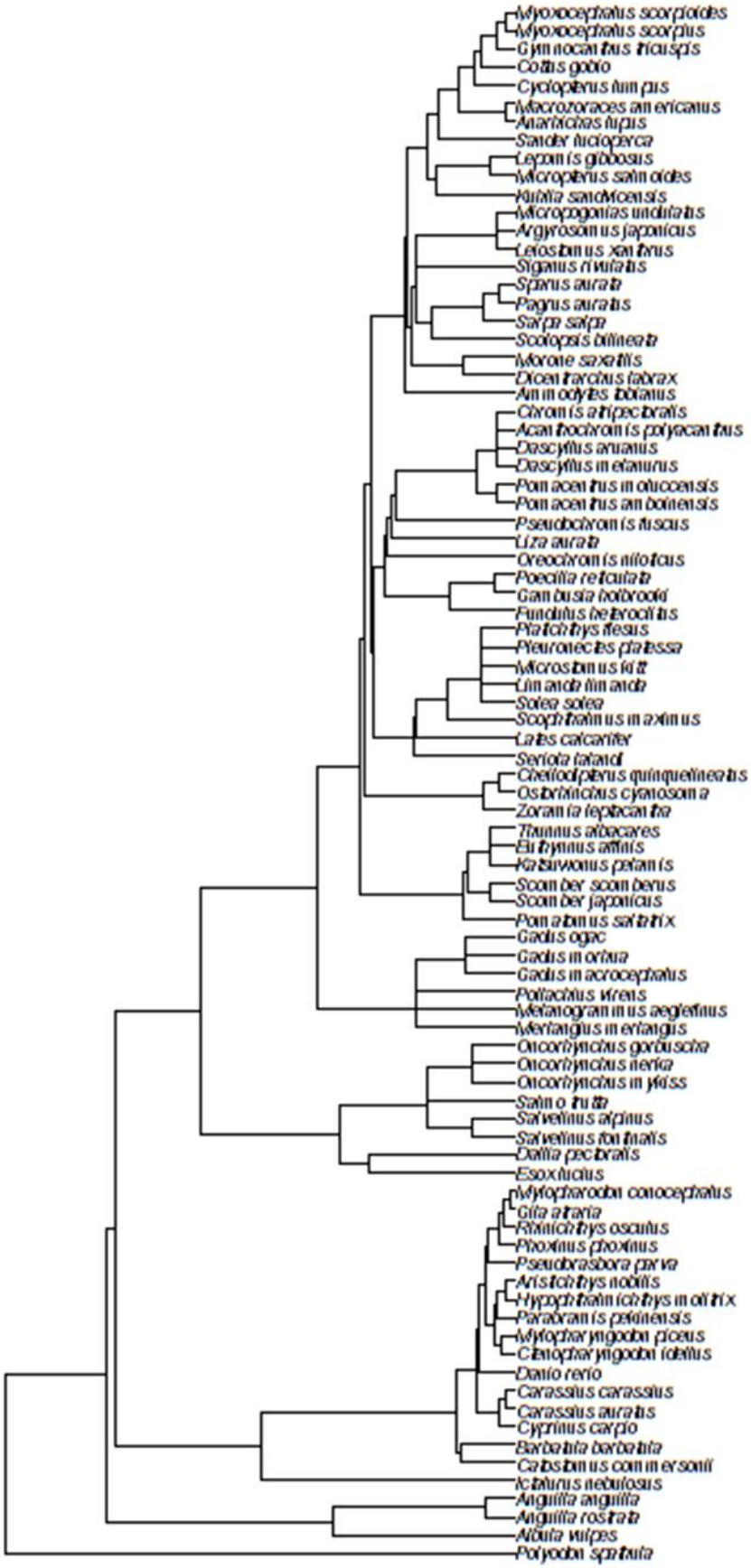



Figure 2.2 Phylogenetic tree generated on the 86 fish species used in PGLS absolute latitude range model. Branch lengths represented in millions of years. The root node was set at around 275 mya (based on two trees obtained by Near et al. (2013) and Wang et al. (2012).

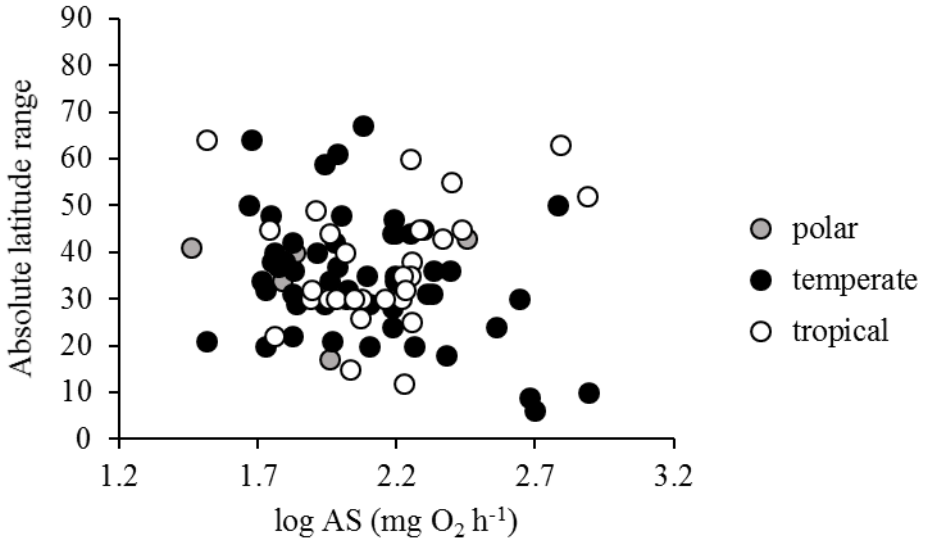



Figure 2.3 Relationship between absolute latitude range (0-90°) and log aerobic scope (in log mg $O_2$ $h^{-1}$) in 86 fish species. For visual representation, data were standardised for body mass and acclimation temperature using residuals from a PGLS multiple regression of log AS versus log body mass and acclimation temperature (logAS = 0.9366*(logmass) + (0.0251*temp) - 0.9373) $R^2$ = 0.883, $p < 0.001$, $\lambda = 0.651$), added to the fitted model value for body mass = 400 g and temperature = 20°C (the mean body mass and acclimation temperature for species used in this study, respectively).

(b) Invasiveness models

The dataset had 59 freshwater fish species from 14 orders and 23 of those were considered to be invasive and 36 native species. Peak aerobic scope performance (log mg $O_2$ $h^{-1}$) was not associated with invasiveness (Table 2.2, Figure 2.4A). Maximum length (log cm) did not show a relationship with invasiveness (Table 2.2, Figure 2.4B).

Within the Cypriniformes order for 27 freshwater fish species, 11 were invasive and 16 native species. In this order for which most of invasive fish species were found, peak aerobic scope (log mg $O_2$ $h^{-1}$) did not explain invasiveness (Table 2.3, Figure 2.5A). However, invasiveness increased with maximum length (log cm) and invasive species are on average longer than native species (Table 2.3, Figure 2.5B).

Table 2.2 Summary of the binominal mixed effect model testing for the effects of aerobic scope (log AS (mg $O_2$ $h^{-1}$)), mass (log g), tested temperature (°C) and maximum length (log cm) on invasiveness (invasive = 1, native = 0) on n = 59 freshwater fish species, with order as random effect to account for phylogenetic relatedness.

| **term** | | **estimate** | **s.e.m** | ***z*** | ***p*** |
|---|---|---|---|---|---|
| intercept | | 0.062 | 1.598 | 0.039 | 0.969 |
| log AS | | 0.783 | 0.951 | 0.824 | 0.410 |
| log mass | | -0.451 | 0.982 | -0.459 | 0.646 |
| temperature | | 0.062 | 0.054 | 1.165 | 0.244 |
| log length | | -0.629 | 0.761 | -0.827 | 0.408 |
| random effect | variance | st.dev. | | | |
| order | 0 | 0 | | | |

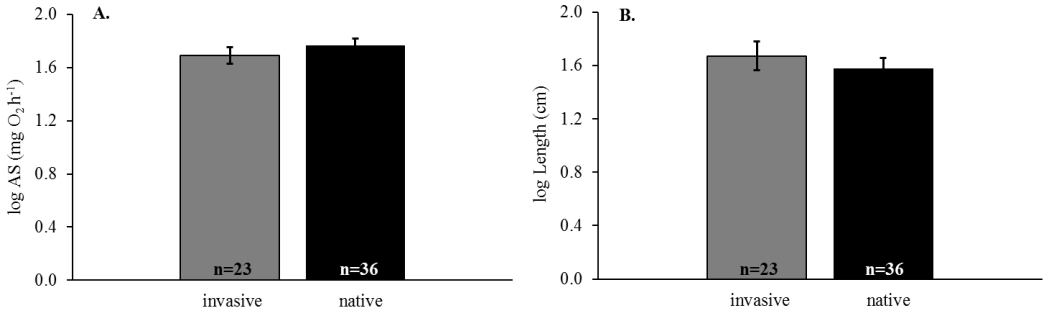



Figure 2.4 Relationship between invasiveness (invasive and native) and log aerobic scope (in log mg $O_2$ $h^{-1}$) in 59 freshwater fish species. For visual representation, data were standardised for body mass and acclimation temperature using residuals multiple regression of log AS versus log body mass and acclimation temperature (logAS = 0.9189*(logmass) - 0.0036*(temp) - 0.2995), $R^2$ = 0.898, p < 0.001, added to the fitted model value for body mass = 200 g and temperature = 22°C (the mean body mass and acclimation temperature for species used in this study, respectively) represented as mean values (± s.e.m). B. Relationship between invasiveness (invasive and native) and log maximum length (in log cm) in 59 freshwater fish species represented as mean values (± s.e.m).

Table 2.3 Summary of the binominal mixed effect model testing for the effects of aerobic scope (log AS (mg $O_2$ $h^{-1}$)), mass (log g), tested temperature (°C) and maximum length (log cm) on invasiveness (invasive = 1, native = 0) on n = 27 freshwater fish species in Cypriniformes order.

| **term** | **estimate** | **s.e.m** | ***z*** | ***p*** |
|---|---|---|---|---|
| intercept | 2.885 | 2.616 | 1.103 | 0.270 |
| logAS | 1.633 | 1.697 | 0.962 | 0.336 |
| logmass | -1.084 | 1.858 | -0.583 | 0.56 |
| temperature | 0.065 | 0.118 | 0.548 | 0.584 |
| loglength | -2.454 | 1.198 | -2.048 | 0.041 |

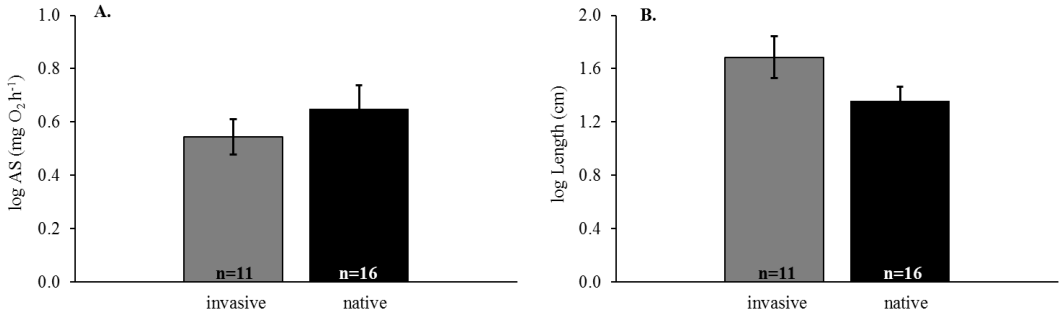



Figure 2.5 A. Relationship between invasiveness (invasive and native) and log aerobic scope (in mg $O_2$ $h^{-1}$) in 27 Cyrpiniformes freshwater fish species. For visual representation, data were standardised for body mass and acclimation temperature using residuals multiple regression of log AS versus log body mass and acclimation temperature (logAS = 0.9669*(logmass) - 0.0028*(temp) + 0.1978), $R_2$ = 0.7961, p < 0.001), added to the fitted model value for body mass = 50 g and temperature = 23°C (the mean body mass and acclimation temperature for species used in this study, respectively) represented as mean values (± s.e.m). B. Relationship between status (invasive and native) and log maximum length (in log cm) in 27 freshwater fish species represented as mean values (± s.e.m).

## 2.5 Discussion

This phylogenetically informed study across 86 species of teleost fishes suggests no link between absolute latitude range and peak aerobic scope performance (Figure 2.3). It therefore appears that an increased peak aerobic scope does not necessarily allow species to inhabit a wider geographical range. These results contrast with the findings of Naya & Bozinovic (2012), who found a positive correlation between the difference between RMR (routing metabolic rate) and SMR (termed the "metabolic scope" by Naya and Bozinovic (2012)) and latitude range across 38 fish species without phylogenetic corrections. They concluded that positive link between RMR-SMR and distribution range in fish may be due

to physiological flexibility while facing different thermal variability trends associated with latitude range. In ectotherms as fish maintaining homeostasis in their baseline or routine metabolic levels (SMR, RMR) is crucial for survival. Showing high plasticity and flexibility responses in RMR or SMR toward thermal changes may give fish the capacity to maintain homeostasis in life sustaining functions in highly thermal variable habitats. As aerobic scope is the whole animal capacity to provide oxygen to a sum of energy demanding activities (e.g. growth, reproduction, locomotion) above the maintenance levels, in a given ecological context (e.g. competition, mating, predation) fish may decide to allocate a sufficient amount of energy and oxygen to a specific performance trait (e.g. growth, reproduction). Additionally, all these underlying performance traits represented by AS, might have different reaction norms towards thermal changes than AS. Therefore, a direct link between AS and distribution range in fish may be biased by underlying energy allocation trade-offs and other biological parameters. However, Payne et al. (2016) found a positive link between optimum temperature for aerobic scope and the highest temperature encountered in the range of nine tropical and temperate marine fish species, suggesting that aerobic scope might set the upper limit at the warmest regions that are habitable by teleost fish. Payne et al. (2016) findings, would indicate that AS is relevant in determining the upper distribution boundaries in fish. Though, it is still not clear how AS is related to overall distribution range. The relevance of AS in current distribution patterns in ectotherms is an ongoing debate and evidences are controversial (Clark et al., 2013; Farrell 2013; Pörtner & Giomi 2013; Pörtner 2014; Wang et al. 2014; Farrell 2016). In this study, I could not find any evidence of peak aerobic scope being relevant in present distribution range across teleost fish species.

There was also no association between invasion success and peak aerobic scope performance (Figure 2.4A; Table 2.2), either in the dataset as a whole or within the cyprinids (Figure 2.5A; Table 2.3). Furthermore, maximum length, here used as proxy for fecundity, did not differ between invasive and native species, but invasive species tend to reach a larger maximum body size within the cyprinids. In fishes, fecundity tends to increase with body size, and so having a larger asymptotic size could give invasive species an advantage if they are able to increase propagule pressure (e.g. number of individuals released or introduced into a novel environment) to facilitate the colonisation and population spread (Lockwood et al., 2005).

Characteristics such as species lifestyle strategy, body size, trophic level and fecundity can all interact to influence the distribution of species and possibly obscure any direct effect of aerobic scope. Fish with different lifestyles (benthic, benthopelagic and pelagic) have different energy requirements (Killen et al., 2016), dispersal capacities and migration patterns (Leggett 1977; Sims et al., 2004; Eliason et al., 2011; Demer et al., 2012). Similarly, complex interactions between aerobic scope and other traits may also modulate range expansion in invasive species, particularly if different traits become more or less important at differing stages of biological invasion (Marchetti et al., 2004; García-Barthou, 2007; Riberio et al., 2008). Life history traits such as fecundity and size at maturity are still lacking for many invasive species and so they were not available to be included in the present analysis, but quantifying these parameters is an important topic for future research (García-Barthou 2007).

As for this particular analysis, the absolute latitude range data used did not give an indication of temporal consistency or change of the given range a species can occupy. Certain fish can perform daily as well as seasonal migrations over an impressive geographical range, whereas others are unable to migrate and inhabit a given range on a continuous temporal basis (Leggett 1977; Sims et al., 2004; Eliason et al., 2011; Demer et al., 2012). Migratory fish, therefore encounter and experience a large variation in thermal conditions as individuals span large latitudinal ranges within a single generation. However, in this study given the latitude data available, I could not distinguish between the range used by a species on regular basis versus the range used during migratory seasons. Consequently, the experienced thermal variations due to these migration trends could not be taken into account and tested for during my analysis.

The aerobic scope data in this dataset may not be the presumed peak AS performance for each given species. As the majority of studies (n = 52) from which AS data were extracted, measured aerobic scope at one test temperature. This might indicate that for some fish species in this dataset, the optimum temperature for AS if it existed, was not defined and therefore the peak performance for AS might not have been achieved. Obviously showing that there is still a need for further research in fish for determining the aerobic scope performance over a large range of temperatures to fulfil this lack of data. As a result, the peak AS data here might not necessarily represent the maximum energetic potential a species can achieve. The fact that AS changes with temperature in many species may also confound attempts to relate AS to latitudinal range via differences in the ability to tolerate

thermal variation. Although there appears to be no relationship between peak AS and the thermal breadth for AS among species (see results Chapter 3), differences in the reaction norm of AS with temperature or the shape of the thermal performance curve for AS may cause differences in the relative rankings of species for AS at different temperatures.

It is also important to note that different fish species may have different requirements for the allocation of aerobic scope for various behaviours and physiological functions. For example, trade-offs in allocation requirements may differ between more active fish species (pelagic species) and benthic sedentary fish species. Benthic fish species might be less constrained by a reduction in aerobic scope due to their less active lifestyle. Nevertheless, in these less mobile benthic species, the energetic investment into the action of digestion (specific dynamic action, SDA) might be higher than that for maximised exercised metabolic rate (MMR). According to the existing evidence in the literature, in most species with either pelagic or benthic lifestyles, it seems unlikely that SDA energetic metabolism is approaching or equal to MMR (see for more details Norin & Clark 2016). The only study showing a higher SDA metabolism under "stable" environmental conditions (e.g. no change in thermal regimes or in dissolved oxygen concentration in the water) as MMR, was in benthic Southern catfish (*Silurus meridionalis*). In this particular case, the peak SDA $MO_2$ consumption (in mg $O_2$ $kg^{-1}$ $h^{-1}$) of Southern catfish was ca. 64% higher than MMR (127.1 ± 8.2 versus 197.9 mg $O_2$ $kg^{-1}$ $h^{-1}$) at 25°C (Fu et al., 2009). Even within a given lifestyle, species may require differing proportions of their aerobic scope for various processes associated with growth, digestion, reproduction, competition, predator avoidance, and foraging. Such differences among species could generate important energy allocation trade-offs that may permit range expansion in specific ecological contexts or environmental conditions that must be considered on a case-by-case basis. This is particularly true when considering the competitive ability of native versus potentially invasive species.

In conclusion, I found no evidence that AS is related to the geographical distribution of fish species or invasion success at the interspecific level. This may suggest that teleost fish species might not be directly constrained by aerobic scope performance when it comes to distribution range and invasion success. It might be possible that fish have the necessary plasticity capacity to adjust their aerobic scope according to energetic needs in order to extend their distribution range. Conversely, it could be likely that fish may encounter underlying physiological constraints masked and obscured by overall aerobic scope

performance. Specific cardio-vascular and respiratory organs (e.g. heart) or internal organelles (e.g. mitochondria) within these organs might reach their performance limits (Iftikar & Hickey 2013) before an actual drop in whole animal aerobic scope performance is observed. It has been suggested that species distribution in fish may be linked to the thermal sensitivities and limits of mitochondrial stability and function in the heart (Iftikar et al., 2014). Further research is need to determine the specific physiological features from molecular to whole organism performance which are important and relevant in species distribution and invasion success.

# Chapter 3: Is there a trade-off between peak performance and performance breadth across temperatures for aerobic scope in teleost fishes?

Collaboration work with Lewis G. Halsey. Published in Biology Letters (28.09.16)

## 3.1 Abstract

The physiology and behaviour of ectotherms are strongly influenced by environmental temperature. A general hypothesis is that for performance traits, such as those related to growth, metabolism, or locomotion, species face a trade-off between being a thermal specialist or a thermal generalist, implying a negative correlation between peak performance and performance breadth across a range of temperatures. Focusing on teleost fishes, I performed a phylogenetically-informed comparative analysis of the relationship between performance peak and breadth for aerobic scope (AS), which represents whole-animal capacity available to carry out simultaneous oxygen-demanding processes (e.g. growth, locomotion, reproduction) above maintenance. Literature data for 28 species indicate that peak aerobic capacity is not linked to thermal performance breadth and that other physiological factors affecting thermal tolerance may prevent such a trade-off from emerging. The results therefore suggest that functional links between peak and thermal breadth for AS may not constrain evolutionary responses to environmental changes such as climate warming.

## 3.2 Introduction

For ectotherms, performance traits related to growth, reproduction and locomotion are often depicted using thermal performance curves (Huey & Stevenson 1979; Angilletta 2009) that illustrate how a trait responds to variation in environmental temperature (Figure 3.1). Evolutionary thermal adaptation (Angilletta 2009) may result in thermal specialists or thermal generalists, performing better over a narrow versus a broad range of temperatures, respectively (Figure 3.1; Gilchrist 1995). Thermal and biochemical constraints on enzyme structure and function and membrane fluidity suggest that adaptations for increased performance at one temperature may cause decreased performance at other temperatures, resulting in a trade-off between peak performance ($P_{max}$) at a thermal optimum and thermal performance breadth ($T_{breadth}$). Due to these potential compromises, previous researchers have suggested that a "jack of all temperatures is a master of none" (Huey & Hertz 1984).

While a trade-off between $P_{max}$ and $T_{breadth}$ is predicted by theory (Levins 1962; Gilchrist 1995), several studies have documented that an increased performance capacity at one temperature does not necessarily lead to reduced performance at other temperatures (Huey & Hertz 1984; Malloy et al., 1994; Purchase et al., 2001; Ojanguren et al., 2001; Wilson et al., 2001). Notably, however, most studies have examined differences in performance among populations of the same species, with few attempts to examine whether a trade-off exists across species. Therefore, it remains unknown whether any trade-off between $P_{max}$ and $T_{breadth}$ generate interspecific constraints on thermal adaptation. Furthermore, most attempts to examine trade-offs between $P_{max}$ and $T_{breadth}$ have focused on isolated components of locomotory performance (e.g. maximum speed, endurance). This approach may, however, fail to detect broader-scale trade-offs in organismal functioning. Aerobic scope (AS), in contrast, is an integrative trait, representing whole-animal cardio-vascular and respiratory capacity to provide oxygen above maintenance requirements, for aerobic activities including growth, locomotion, and reproduction (Fry 1971; Clark et al., 2013). In ectotherms, AS generally increases with temperature until $T_{opt}$ and then usually decreases with further warming (Farrell 2016, but see Lefevre 2016 for exceptions), potentially providing a composite measure to examine thermal sensitivity of whole-animal aerobic performance. Aerobic scope is also ecologically relevant and has been related to geographical distribution (Payne et al., 2016), the capacity to cope with environmental stressors (Claireaux & Lefrancois 2007) and competitive ability (Seth et al., 2013). Additionally, species with a higher AS tend to be more active and athletic (Killen et al.,

2016), presumably because increased locomotion requires a greater allocation of oxygen to skeletal muscles (Farrell 2016). If there is indeed a trade-off between $P_{max}$ and $T_{breadth}$, then ectothermic species that experience selection for increased peak AS to facilitate foraging, predator avoidance, or migration may conversely have a reduced $T_{breadth}$ (Farrell 2016).

I investigated the relationship between $P_{max}$ and $T_{breadth}$ for AS across 28 species of teleost fish, to determine the extent to which adaptation for performance at a particular optimal temperature may impose constraints on performance across a range of temperatures. I focused on fishes because they are a diverse taxon that experiences shifts in thermal regimes over varying timescales. It has also been proposed that AS may influence the ability of fishes to respond to climate change (Pörtner & Farrell 2008).

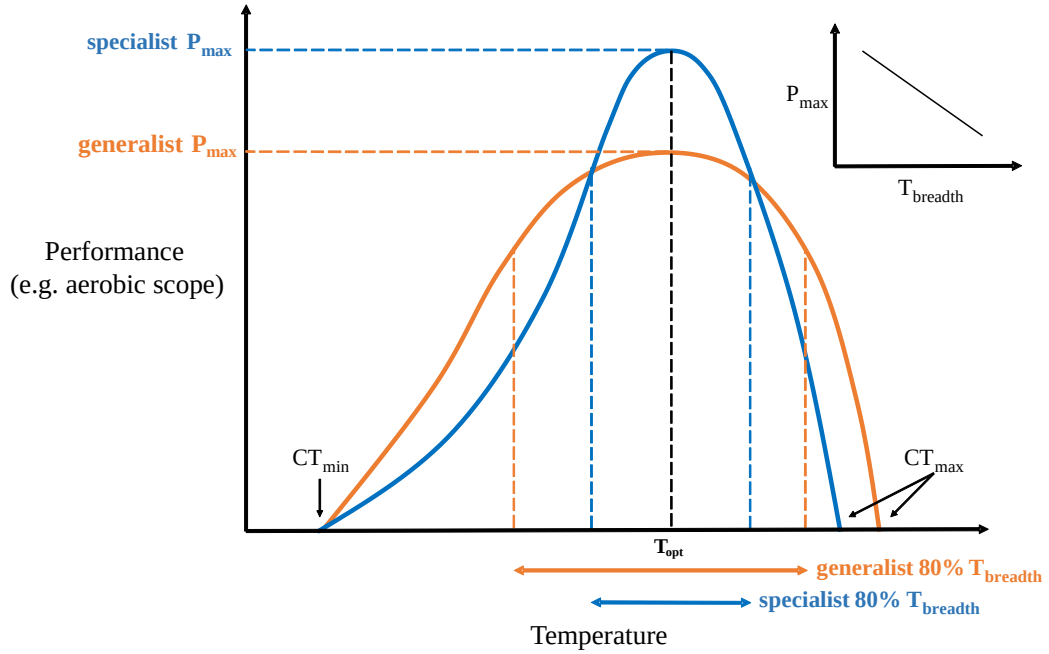



Figure 3.1 Theoretical thermal performance curves illustrating thermal specialists (higher peak performance, in blue) and thermal generalists (higher performance breadth, in orange). Specialists have a higher peak performance ($P_{max}$) at their optimum temperature ($T_{opt}$). Generalists have a lower $P_{max}$ but a wider breadth of temperatures over which they perform normally ($T_{breadth}$, here defined as the range of temperatures allowing 80% of $P_{max}$). Critical minimum and maximum thermal limits ($CT_{min}$ and $CT_{max}$, respectively) occur where performance equals zero. Inset: predicted negative correlation between $P_{max}$ and $T_{breadth}$ if there is a trade-off between being a thermal specialist and a thermal generalist.

## 3.3 Methods and Materials

Data for the aerobic scope (AS) of fish, calculated as the difference between standard and maximum metabolic rates (SMR and MMR), were extracted from the literature where they were available at three or more temperatures (n = 28). A Gaussian model was fitted to the relationship between AS and temperature for each species to produce a thermal performance curve for each study (Angilletta 2006). Peak AS ($P_{max}$) was defined as the

highest value for AS along the thermal performance curve. The optimum temperature ($T_{opt}$) was the temperature corresponding to $P_{max}$ (Fry 1971; Huey & Stevenson 1979). I used one dataset per species; when more than one dataset was available, I used the dataset which measured AS at the highest number of temperatures. If multiple datasets were available for a species within a given study, I used that which gave the highest value for $P_{max}$. Performance breadth ($T_{breadth}$) was the range of temperatures over which a species maintained at least 80% of peak AS (Frisk et al., 2012). As the 80% of $P_{max}$ designation for $T_{breadth}$ range is somewhat arbitrary, I also examined 60, 70 and 90% thresholds for $T_{breadth}$. Data for an additional 15 species were not included because either: a) AS did not increase or decrease appreciably over the temperatures tested (i.e. the performance "curve" was flat; 2 species); b) AS decreased with no obvious peak across temperatures tested in the study, perhaps because the lowest temperature in the study was above $T_{opt}$ (3 cases); c) AS increased with no obvious peak across temperatures tested in the study, perhaps because the highest temperature in the study was below $T_{opt}$ (9 cases); or d) AS continued to increase with temperature until $CT_{max}$ (1 case).

Data were analysed using the phylogenetic generalised least squares (PGLS) method applying a phylogeny generated from the comprehensive tree of life (Hinchliff et al., 2015) (Figure 3.3 for more details on statistical analysis, including phylogenetic tree). Log $P_{max}$ (mg $O_2$ $h^{-1}$) was the response variable with $T_{breadth}$, $T_{opt}$, lifestyle (benthic, benthopelagic and pelagic) and log body mass (g) as explanatory variables. Six studies measured AS in fish acutely exposed to each temperature; I therefore constructed a separate model using only studies in which fish were thermally acclimated (n = 20, Table 3.2). Model residuals were checked for normality and homogeneity of variance. The significance level of all tests was $p = 0.05$.

## 3.4 Results

PGLS analysis revealed no relationship between $P_{max}$ and $T_{breadth}$ regardless of the threshold used to define $T_{breadth}$ (Figure 3.2A, model details for 80% $T_{breadth}$ threshold in Table 3.1; for 60%, p = 0.188; 70%, p = 0.368; 90%, p = 0.200; Table 3.3-3.5). $P_{max}$ increased with $T_{opt}$ (Figure 3.2B; PGLS, effect of $T_{opt}$, t = 4.240, p < 0.001). Explanatory variables explained 92.2% of variation in $P_{max}$ ($R^2$). Trends were identical when PGLS models were performed using studies with acclimated animals only (Table 3.2, PGLS, effect of $T_{breadth80\%,}$ t = -0.752, p = 0.466).

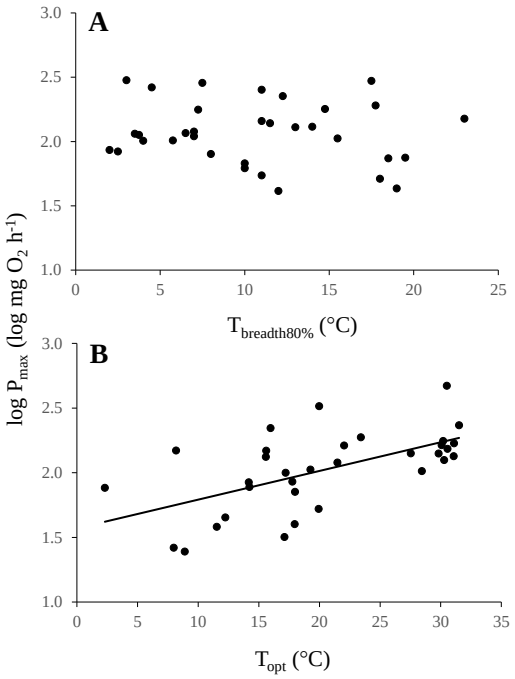



Figure 3.2 A) Relationship between log-transformed peak aerobic scope ($P_{max}$) and thermal performance breadth ($T_{breadth}$). For visual representation, data were standardised for body mass and $T_{opt}$ using residuals from a PGLS multiple regression of log $P_{max}$ versus log body mass and $T_{opt}$ (log $P_{max}$ = 1.0013(log mass) + 0.0285($T_{opt}$) – 0.9778; p < 0.001, $R^2$ = 0.924), added to the fitted model value for body mass = 300 g and $T_{opt}$ = 20°C (the mean body mass and $T_{opt}$ for species used in this study, respectively). B) Relationship between $P_{max}$ and optimum temperature ($T_{opt}$). For this panel, $P_{max}$ was standardised to a body mass of 300 g using residuals of a PGLS linear regression of log $P_{max}$ versus log body mass (log $P_{max}$ = 0.9409(log mass) - 0.3227; p < 0.001; $R^2$ = 0.864). In both panels, each point represents one species (n = 28).

Table 3.1 Summary of the PGLS model testing for the effects of aerobic scope breadth ($T_{breadth80\%}$ °C), optimal temperature ($T_{opt}$ °C) and body mass (log g) on $P_{max}$ (log mg $O_2$ $h^{-1}$). $R^2$ = 0.937, $F_{5, 21}$ = 62.59, p < 0.001, n = 28 species, λ = 0.70. For lifestyle categorisation, the reference category is 'benthic'.

| **term** | **estimate** | **s.e.m** | ***t*** | ***p*** |
|---|---|---|---|---|
| intercept | -1.000 | 0.252 | -3.971 | <0.001 |
| $T_{breadth80\%}$ | -0.01 | 0.008 | -1.249 | 0.226 |
| $T_{opt}$ | 0.03 | 0.007 | 4.240 | <0.001 |
| log mass | 1.01 | 0.066 | 15.387 | <0.001 |
| **lifestyle** | | | | |
| benthopelagic | 0.072 | 0.110 | 0.660 | 0.516 |
| pelagic | 0.246 | 0.117 | 2.095 | 0.049 |

Table 3.2 Summary of the phylogenetic least squares regression model testing for the effects of thermal performance breadth for aerobic scope (80 % of $P_{max}$), optimal temperature ($T_{opt}$), body mass (log g) and lifestyle (benthic, benthopelagic, or pelagic) on maximum aerobic scope ($P_{max}$; log mg $O_2$ $h^{-1}$). For lifestyle categorisation, the reference category is 'benthic'. This analysis only used studies in which fish were acclimated to each experimental temperature. $R^2$ = 0.94, $F_{5, 13}$ = 40.76, p < 0.001, n = 20 species, λ = 0.659.

| **term** | **estimate** | **s.e.m** | ***t*** | ***p*** |
|---|---|---|---|---|
| intercept | -0.795 | 0.268 | -2.964 | 0.011 |
| $T_{breadth}$ | -0.006 | 0.008 | -0.752 | 0.466 |
| $T_{opt}$ | 0.027 | 0.008 | 3.35 | 0.005 |
| log mass | 0.923 | 0.084 | 10.956 | <0.001 |
| **lifestyle** | | | | |
| benthopelagic | 0.021 | 0.103 | 0.204 | 0.841 |
| pelagic | 0.155 | 0.17 | 0.91 | 0.379 |

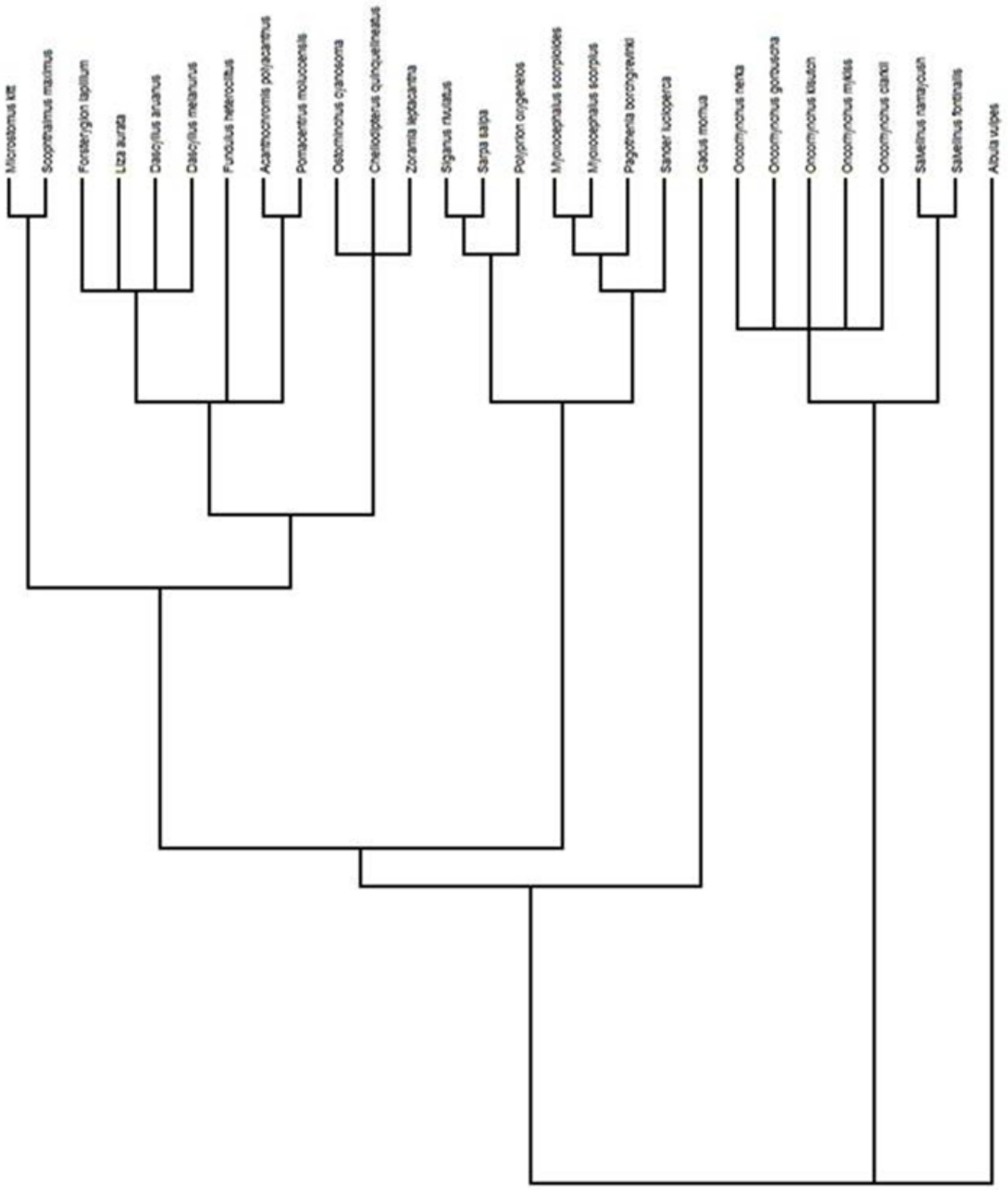



Figure 3.3 Phylogenetic tree generated on the 28 fish species used in PGLS trade-off model. Data were analysed using the phylogenetic generalised least squares (PGLS) method (Grafen 1989; Garland & Ives 2000) with the 'caper' package (Orme 2013) in R, applying a phylogeny generated from the comprehensive tree of life (Hinchliff et al., 2015) using the 'rotl' package (Michonneau et al., 2015). The branch lengths in the tree were estimated using Grafen's arbitrary branch lengths transformation (Grafen 1989) (branch lengths set to a length equal to the number of descendant tips minus one). A measure of phylogenetic correlation, λ (Freckleton et al., 2002) was estimated by fitting PGLS models with different values of λ and finding the value that maximises the log likelihood. Thus the degree to which trait evolution deviates from Brownian motion (λ = 1) was determined by modifying the covariance matrix using the maximum-likelihood value of λ, which is a multiplier of the off-diagonal elements of the covariance matrix (i.e. those quantifying the degree of relatedness between species).

Table 3.3 Summary of the phylogenetic least squares regression model testing for the effects of thermal performance breadth for aerobic scope (60 % of $P_{max}$), optimal temperature ($T_{opt}$), body mass (log g) and lifestyle (benthic, benthopelagic, or pelagic) on maximum aerobic scope ($P_{max}$; log mg $O_2$ $h^{-1}$). $R^2$ = 0.93, $F_{5, 21}$ = 55.19, p < 0.001, n = 28 species, λ = 1. For lifestyle categorisation, the reference category is 'benthic'.

| **term** | **estimate** | **s.e.m** | ***t*** | ***p*** |
|---|---|---|---|---|
| intercept | -0.856 | 0.299 | -2.862 | 0.009 |
| $T_{breadth}$ | -0.007 | 0.005 | -1.362 | 0.188 |
| $T_{opt}$ | 0.025 | 0.007 | 3.533 | 0.002 |
| log mass | 0.975 | 0.065 | 15.021 | <0.001 |
| **lifestyle** | | | | |
| benthopelagic | 0.088 | 0.081 | 1.093 | 0.287 |
| pelagic | 0.281 | 0.105 | 2.676 | 0.014 |

Table 3.4 Summary of the phylogenetic least squares regression model testing for the effects of thermal performance breadth for aerobic scope (70 % of $P_{max}$), optimal temperature ($T_{opt}$), body mass (log g) and lifestyle (benthic, benthopelagic, or pelagic) on maximum aerobic scope ($P_{max}$; log mg $O_2$ $h^{-1}$). $R^2$ = 0.935, $F_{5, 21}$ = 60.86, p < 0.001, n = 28 species, λ = 0.684. For lifestyle categorisation, the reference category is 'benthic'.

| **term** | **estimate** | **s.e.m** | ***t*** | ***p*** |
|---|---|---|---|---|
| intercept | -1.01 | 0.255 | -3.955 | <0.001 |
| $T_{breadth}$ | -0.006 | 0.006 | -0.919 | 0.368 |
| $T_{opt}$ | 0.03 | 0.007 | 4.147 | <0.001 |
| log mass | 1.005 | 0.066 | 15.177 | <0.001 |
| **lifestyle** | | | | |
| benthopelagic | 0.067 | 0.112 | 0.596 | 0.558 |
| pelagic | 0.248 | 0.120 | 2.065 | 0.052 |

Table 3.5 Summary of the phylogenetic least squares regression model testing for the effects of thermal performance breadth for aerobic scope (90 % of $P_{max}$), optimal temperature ($T_{opt}$), body mass (log g) and lifestyle (benthic, benthopelagic, or pelagic) on maximum aerobic scope ($P_{max}$; log mg $O_2$ $h^{-1}$). $R^2$ = 0.929, $F_{5, 21}$ = 54.92, p < 0.001, n = 28 species, λ = 1. For lifestyle categorisation, the reference category is 'benthic'.

| term | estimate | s.e.m | *t* | *p* |
|---|---|---|---|---|
| intercept | -0.858 | 0.3 | -2.864 | 0.009 |
| $T_{breadth}$ | -0.015 | 0.011 | -1.324 | 0.2 |
| $T_{opt}$ | 0.025 | 0.007 | 3.509 | 0.002 |
| log mass | 0.975 | 0.065 | 14.98 | <0.001 |
| **lifestyle** | | | | |
| benthopelagic | 0.087 | 0.081 | 1.078 | 0.293 |
| pelagic | 0.281 | 0.105 | 2.673 | 0.014 |

## 3.5 Discussion

I found no evidence of a trade-off between $T_{breadth}$ and $P_{max}$ for AS across teleost fishes. Evolutionary and plastic changes to peak performance for AS may not necessarily lead to reduced performance over a broader range of temperatures and so there may not be differentiation along a thermal specialist/generalist continuum with respect to AS in teleost fishes. Investment in factors such as gill surface area, heart pumping capacity, tissue vascularisation and mitochondrial density should all act to increase $P_{max}$ (Norin & Malte 2012; Killen et al., 2016). The results here suggest that these features can also increase AS at other temperatures, particularly if compensatory mechanisms allow for plasticity in each in response to temperature (e.g. change in the concentration or isoforms of aerobic enzymes, Prosser 1991). It has been proposed that AS may constrain geographical distributions of aquatic ectothermic species in the face of a warming climate and other aspects of environmental changes (Pörtner & Farrell 2008). The findings here suggest that the evolutionary potential of $P_{max}$ is not constrained by prior adaptation for a wider $T_{breadth}$, or *vice versa*, depending on the degree of genetic correlation between $P_{max}$ and $T_{breadth}$. For example, stenothermal species, which may have experienced relaxed selection for $T_{breadth}$, may be able to readily evolve a broader performance breadth or shift $P_{max}$ in response to changing thermal regimes, at least for AS. There are also other factors influencing plastic

and evolutionary responses to thermal regimes that perhaps override or obscure links between these two traits $P_{max}$ and $T_{breadth}$ for AS. For example, a decreased $T_{breadth}$ for AS may not compromise fitness to the same extent as a reduced ability to adjust cellular membrane fluidity in response to thermal variation (Cossins & Prosser 1978). This could preclude a negative correlation between $P_{max}$ and $T_{breadth}$ for AS from arising across species through evolutionary processes. It should also be noted that there appears to be several fish species which may not show a decline in AS with increasing temperature before reaching $CT_{max}$ (Lefevre 2016). In such species, $P_{max}$ must also not be constrained by performance breadth for AS.

This phylogenetically-informed analysis spans a variety of species with varying lifestyles. Still, complex interactions among thermal history, body size, lifestyle, and evolutionary history could also mask a trade-off between $P_{max}$ and $T_{breadth}$ for AS. Across fishes, AS differs among benthic, benthopelagic, and pelagic species (Killen et al., 2016), and so it is also possible that selection on AS to support locomotory capacity may outweigh thermal effects on $P_{max}$. This interpretation is supported by the observation that pelagic species had a higher $P_{max}$ in the current study (Table 3.1). It must also be considered that species may differ in the percentage of $P_{max}$ most relevant for defining $T_{breadth}$ (Farrell 2016). For example, species that perform long migrations might require a higher proportion of $T_{breadth}$ to maximise lifetime fitness as compared to more sedentary species.

$P_{max}$ was higher in species with a higher $T_{opt}$, providing support for the "hotter is better" model (Angilletta et al., 2010). AS in fishes generally increases with temperature until $T_{opt}$ and then decreases as temperature increases further (though see Lefevre 2016). It is unlikely that species would live in habitats with temperatures much higher than $T_{opt}$, given that thermal performance curves can be asymmetrical, with performance dropping more steeply above $T_{opt}$ (Farrell 2016). To date, most studies have not measured AS at sufficient temperature points to permit complex asymmetrical modeling (Angilletta 2006) and so we have little understanding of how curve asymmetry may be linked with thermal specialisation for AS. This may be especially relevant for the many tropical fishes that have a decreased thermal window between $CT_{min}$ and $CT_{max}$ (Sunday et al., 2011). Future studies examining changes in AS with temperature in ectotherms should perform measurements across as many temperatures as possible to permit complex modelling of asymmetrical performance curves.

# Chapter 4: Metabolic and behavioural responses to thermal variation in an invasive and native freshwater fish species

Currently in preparation for submission to Journal of Experimental Biology

## 4.1 Abstract

Biological invasion is one of the major ecological threats to biodiversity with fish being one of the most introduced taxa worldwide. Shifts and changes in fish communities are expected as well with increasing temperatures, particularly in the smallest freshwater bodies. Due to changing thermal conditions in rivers and streams, the establishment and spread of invasive fish species may be facilitated and result in competition with native fish or in their displacement into less suitable habitats. The aim of this study was to compare physiological and behavioural responses of a native fish species, stone loach (*Barbatula barbatula*), and an invasive fish species, bullhead (*Cottus gobio*), across their current thermal range (summer temperatures: 13-21°C) in order to explain the invasion success of bullheads in Scottish rivers. I compared metabolic traits in both species during acute and acclimated thermal trials and behavioural traits (activity levels and temperature preference). Both species had similar metabolic responses with minor changes over tested thermal range 13-21°C. Nevertheless, bullheads showed lower maximum metabolic rate (MMR) and aerobic scope (AS) values than stone loaches over all temperatures tested. Bullheads were also less active and preferred colder temperatures (17.5-19°C) than stone loaches (21-22.4°C). The invasion success of bullheads might therefore be due to the combined effects of physiological and behavioural attributes. Having reduced aerobic scope and limited activity levels are less costly energetically and may give an advantage during harsh unstable conditions. Additionally, due to sedentary lifestyle, bullheads might have more flexibility in their energy allocation budget. In theory, bullheads may therefore also have more energy available to invest in other activities such as aggressive behaviour and competitive interactions to defend their home range against native stone loaches. These interactions may have led to the displacement of native stone loaches into less suitable habitats.

## 4.2 Introduction

Invasive species are a major threat to biodiversity and structure of ecological communities (Moyle 1986; Kitchell et al., 1997; Gozlan et al., 2005; Blanchet et al., 2007). Despite the increased introduction rate of non-native aquatic species into novel environments (Sala et al., 2000), not all species become successful invaders. Several life history traits, such as short lifespan and high reproductive output, for example, are known to be relevant in the invasion process (Ehrlich 1984; Moyle 1986; Sakai et al., 2001). However, studies considering physiological traits involved in the process of biological invasion are still limited (Cooke et al., 2013; Lennox et al., 2015). Physiological traits could be key factors in the spread of invasive species, particularly the internal underlying mechanisms that modulate an organism's response to environmental changes (Kelley 2014).

In aquatic habitats, climate change is suspected to facilitate the invasion process for some species by changing thermal regimes (Rahel & Olden 2008) and creating new suitable habitats for non-native warm water species (Lehtonen 1996). Temperature is one of the major abiotic factors influencing physiological processes in ectotherms. Aerobic scope (AS), for example, is the absolute difference between maximum metabolic rate (MMR) and standard metabolic rate (SMR; minimum of energy required to sustain life), and corresponds to the whole-animal cardio-vascular and respiratory capacity to provide oxygen to simultaneous energy demanding processes (e.g. growth, locomotion and reproduction) above maintenance requirements (Fry 1947; Clark et al., 2013). In many species, AS increases with temperature, peaks at an optimum temperature and drops with further warming (Fry 1971; Pörtner 2006). Aerobic scope is thought to be relevant in several ecological processes (e.g. responses to climate change, Pörtner & Knust 2007; interspecific competition, Seth et al., 2013; geographical distribution, Payne et al., 2016) and can be used to assess an organism's thermal sensitivity.

A few recent studies have shown differing metabolic responses to temperature changes for invasive versus native ectotherms. For example, invasive gammarid species can have a lower SMR than native species across a range of temperatures, possibly giving them an energetic advantage due to reduced maintenance requirements (Maazouzi et al., 2011, Becker et al., 2016). In other cases, native and invasive species have differing thermal ranges over which their aerobic metabolism is optimised. For example, some invasive species optimise aerobic metabolism at colder temperatures than native species (Maazouzi et al., 2011) or vice versa (Marras et al., 2015). In theory, an invasive species with a higher

AS or lower SMR over a broad temperature range might be less constrained by thermal changes (chronic or acute changes) compared to a native species with a comparatively low AS or high SMR. Interspecific differences in metabolic traits and their patterns of change with environmental context (e.g. temperature, resources availability) might influence the success of invasive species.

Behavioural traits may also play a determining role in the invasion success of an introduced species. Within species, including those which are known to be invasive, more active individuals explore and spread faster into novel areas (Reahe & Sih 2004; Cote et al., 2010; Lopez et al., 2012; Myles-Gonzalez et al., 2015). Nevertheless, increased activity comes with increased energetic costs and, at least in some contexts, is linked to resting metabolic rate (Myles-Gonzalez et al., 2015) or AS. In benthic fish species, such as round goby (*Neogobius melanostomus*), it has been shown that individuals with higher resting metabolic rate (RMR) were more active than individual with lower RMR (Myles-Gonzalez et al., 2015). Whereas, in juvenile crucian carps (*Carassius auratus*), no relationship between standard metabolic rate and activity levels could be found (Zeng et al., 2016). As for the relationship between AS and activity levels in benthic fish species, there seems to be a clear lack of information in the literature. This indicates a need for more investigations in this research venue. Activity or any behavioural trait for that matter can also be profoundly affected by temperature. For ectotherms in particular, warmer temperatures generally cause an increase in activity depending on the thermal tolerances of the species (Nakayama et al., 2015). Correspondingly, the dispersal potential of invasive species may increase with rising temperatures via an increase in overall activity and levels of exploration among individual animals.

The temperature preference of species might also be relevant in the invasion process due to links with habitat choice (Freitas et al., 2015). Temperature preference is species-specific and has been well studied in various fish species (Coutant 1977; Jobling 1981; Kita et al., 1996; Khan & Herbert 2012, Norin et al., 2014). Within species, it has been suggested that AS may be linked to thermal preference (Killen 2014). Knowledge is still limited regarding how differences in temperature preference between native and invasive species may affect the invasion process and success of invasive species. Theoretically, differences in preferred temperatures between native and invasive species could lead to shifts in the relative distribution and competitive ability of individuals and species as thermal regimes warm, potentially allowing invasive species to dominate areas previously inhabited by native species.

In this study I compared the metabolic rate, SMR, AS, and spontaneous activity between a native freshwater fish species, stone loach (*Barbatula barbatula,* Linnaeus 1758) and an invasive fish species, bullhead (*Cottus gobio,* Linnaeus 1758) at different temperatures. Stone loaches are native to Scottish freshwater river ecosystems whereas bullheads are an introduced species that is considered to be invasive (Maitland & Campbell 1992; McColl et al., 2009; Yeomans & Jennings 2013). Both species are benthic bottom-dwelling, occupy the same ecological niche, and have similar distribution ranges (e.g. England and Scotland (Wheeler 1969), Northeast Europe (Prenda et al., 1997a)). However, there is evidence that within specific habitats there is mutual avoidance and negative correlation in abundance between bullheads and stone loaches in some streams (Smyly 1957; Mann 1989). The overall aim of this study was to examine differences in thermal sensitivity between invasive bullheads and native stone loaches by looking into physiological and behavioural traits which might be relevant in the invasion process. Specific objectives were to: 1) quantify interspecific differences in metabolic (SMR, MMR and AS) and behavioural traits (activity and temperature preference) in fish acclimated and acutely exposed to a range of temperatures; and 2) test for association between physiological and behavioural traits within both species. I hypothesised that bullheads might have a different thermal metabolic phenotype with a higher AS over all tested temperature compared to stone loaches. The invasion success of bullheads in Scottish rivers might have resulted from these expected behavioural and physiological differences. This study provides insight in physiological and behavioural differences between native versus invasive species and increases our understanding of the success of an invasive species outside its native geographical range.

## 4.3 Methods and Materials

### 4.3.1 Animals and holding conditions

Fish were caught in two different rivers in Scotland, United Kingdom. Adult stone loaches were obtained from two sites, one site at Rotten Calder Water and one site at White Cart catchment near Pollock. Adult bullheads were caught at the White Cart catchment near Pollock. Stone loaches obtained from the wild at Rotten Calder Water have never been in contact with invasive bullhead whereas the White Cart bullheads population are known to co-habit with stone loaches in this river catchment (William Yeomans, pers. comments). Fish were transported by road to Institute of Biodiversity, Animal Health and Comparative

Medicine (IBAHCM) in Glasgow, Scotland, United Kingdom, where they were held in 92 L (L = 620 mm, W = 620 mm and D = 240 mm) tanks each separately supplied with a continuous flow of dechlorinated, UV-sterilised freshwater at 17°C. The photoperiod was a 12:12 light:dark cycle. Fish were fed daily *ad libitum* with frozen bloodworms and once a week with chunks of small fish to avoid a lack of protein in their diet. Three months before the experiments all fish, 21 stone loaches and 30 bullheads, were lightly anaesthetised using benzocaine and marked using coloured VIE elastomer tags (Northwest Marine Technology Inc.) to allow for individual identification during upcoming experiments and allocated to 2 or 3 stone loaches per tank (47 L, L = 520 mm, W = 380 mm and D = 240 mm) and 6 bullheads per tank (47 L, L = 520 mm, W = 380 mm and D = 240 mm or 30 L, L = 380 mm, W = 380 mm and D = 210 mm). Each of the holding tanks contained gravel substrate, plastic plants and circular plastic pipes to provide shelter for the fish. All experiments conducted during this study were in compliance with Home Office legalisation (Project Licence number: 60/4461) in United Kingdom.

### 4.3.2 Respirometry setup

Measures of metabolic rate were estimated by measuring the oxygen uptake rate ($MO_2$) of individual fish using intermittent-flow respirometry following the practices described by Steffensen (1989) and Clark et al. (2013). Eight glass respirometers (163 ml) were submerged in a darkened experimental tank of 93 L (L = 780 mm, W = 570 mm, D = 210 mm) with air-saturated water. Temperature within the experimental tank was controlled by a thermostatic water bath reservoir connected to the experimental tank by a thermo-regulator (TMP-REG system, Loligo Systems, Tjele, Denmark). To maintain a good mixing of water and avoid an oxygen gradient in the respirometry chambers, a continuous water flow (100 ml/min) was induced by a peristaltic pump (Masterflex L/S 100 RPM, Cole-Parmer). Two Eheim flush pumps connected to an automated digital recycling timer (MFRT-1, Superpro Hydroponics) flushed the chambers for 3 min every 13 min with surrounding oxygenated water from the experimental tank. A UV filter was connected to the experimental tank to reduce bacterial respiration during the trials. Oxygen concentrations in chambers were measured every two seconds using two Firesting 4 channel oxygen meters and associated sensors (PyroScience GmbH, Aachen, Germany). During the off flushing cycle of 10 min, $MO_2$ was measured by the decline in oxygen concentration in each chamber. During trials, the experimental bath was covered with black plastic to avoid any external disturbances. Additionally, the sides of the respirometry

chambers were covered with opaque material so fish could not come into visual contact (see Photos A., B.).

### 4.3.3 Respirometry protocol: acute thermal exposures

In January 2014, 8 stone loaches (7.02 ± 0.91 g) and 5 bullheads (6.64 ± 0.86 g) were randomly selected from the holding tanks (17°C) and used for acute thermal exposed trials. They were food-deprived for 48 h before the experiments. Individuals from both species were tested over five temperatures 13, 15, 17, 19 and 21°C representing the ecologically relevant range of temperatures experienced in the wild during the summer season in Scottish rivers. The acute thermal exposure protocol consisted of two cohort groups, one (increasing temperature) cohort starting at 13° and was gradually increased to 15, 17, 19° C to end up at 21°C and a second (decreasing temperature) starting at 21°C and was gradually decreased to 19, 17, 15°C and to end up at 13°C with 4 stone loaches and 2 or 3 bullheads per cohort. A separated experimental tank was used for the acute thermal trials (L = 505 mm, W = 340 mm, D = 220 mm; Vol = 38 L) with a water depth of 10 cm. The tank was divided in two sections by a divider to avoid contact between the two species. Temperature control setup was the same as described above and temperature was increased or decreased at a rate of 2°C $h^{-1}$ (Elliott et al., 1994, 1995; Norin et al., 2014). This rate of thermal change was used as well in other studies conducted on bullheads and stone loaches (Elliott et al., 1994, 1995) and did not induce physiological stress responses in both species. The thermal range and rate of change used in this study on these two fish species corresponded to the natural thermal conditions experienced during the summer seasons in the Clyde river catchments. Feeding did not cease in both species over the tested thermal range (13-21°C; for adult stone loaches 28°C and for adult bullheads 26.5°C; Elliott et al., 1994, 1995). After reaching one of the five tested temperatures, fish were transferred individually in a circular tank (D = 50 cm) with a water depth of 10 cm and manually chased until exhaustion (Killen et al., 2012; Clark et al., 2013; Killen 2014; Killen et al., 2015, see Appendix A2) to obtain maximum metabolic rate (MMR). Immediately after complete exhaustion (occurring after 2 min 36 s on average for the bullheads and 2 min 10 s on average for the stone loaches), fish were placed individually in respirometers. This chase protocol was chosen as both of these fish species are benthic and unable to perform sustained $U_{crit}$ swimming protocol (Reidy et al., 1995; Killen et al., 2007; Clark et al., 2013). Oxygen consumption rate during the first 5 min closed time was considered to be MMR (in mg $O_2$ $h^{-1}$). The fish were then left undisturbed for 24 h during which $MO_2$ was measured as previously described. Standard metabolic rate (SMR, in mg $O_2$ $h^{-1}$) was taken

as the 10% percentile of measures throughout this period (Dupont-Prinet et al., 2010; Killen et al., 2012; Killen 2014), excluding the first 5 h when the oxygen consumption was still elevated due to handling stress (Killen 2014). To account for bacterial respiration during the trials, background consumption was measured before and after each trial in each of the respirometry chambers. This protocol was repeated for all five tested temperatures (13, 15, 17, 19 and 21°C) for both cohort groups.

### 4.3.4 Respirometry protocol: acclimated thermal exposures

Five to six bullheads (5.63 ± 0.33 g) and five to six stone loaches (6.44 ± 0.54 g) were acclimated to three temperatures 13, 18 and 21°C (± 0.5°C) for two weeks. To maintain the holding tanks at warmer temperatures (18°C and 21°C), heaters (NeoHeater, AquaEl IP68 75W) were installed in the holding tanks. Temperatures were recorded and monitored on a daily basis. A weekly water change was performed to limit nitrogen waste. After the two weeks of acclimation, fish were starved for 48 h before SMR and MMR measurements at the acclimated temperatures using the same respirometry protocol as described for the acute thermal trials (see Photos A., B.).

### 4.3.5 Activity and temperature preference trials

After the acclimated $MO_2$ measurements, fish (5-6 stone loaches and 5-6 bullheads per temperature treatment) were individually measured for daily (night and morning) activity levels and preferred temperature at the three acclimation temperatures (13°C, 18°C and 21°C). These experiments were performed in a shuttle-box system (Loligo Systems, Tjele, Denmark) consisting of a figure 8-shaped acrylic tank with circular sections (diameter 30 cm on each side) connected by a small channel (L = 7 cm and W = 5.5 cm). One side was designed to be the warm side and the other to be the cold one. To control the temperature within each chamber, buffer tanks were installed externally to the system and connected to heating and cooling reservoirs. The heating reservoir was set up with four heater units (NeoHeater, AquaEl) and the cooling reservoir was kept cooled with a chiller unit. Eheim pumps pumped water from the buffer tanks through steel coils placed in the two reservoirs to change temperature as required during the experiment. On each side, temperature was continuously measured with in-line external temperature probes. These probes were connected to a computer-driven temperature controller and data acquisition system (DAQ-M; Loligo Systems). The control of temperature within each side was performed using software (SHUTTLESOFT; Loligo Systems) connected to a camera (uEye; Imaging Development Systems GmbH, Obersulm, Germany) mounted above the experimental

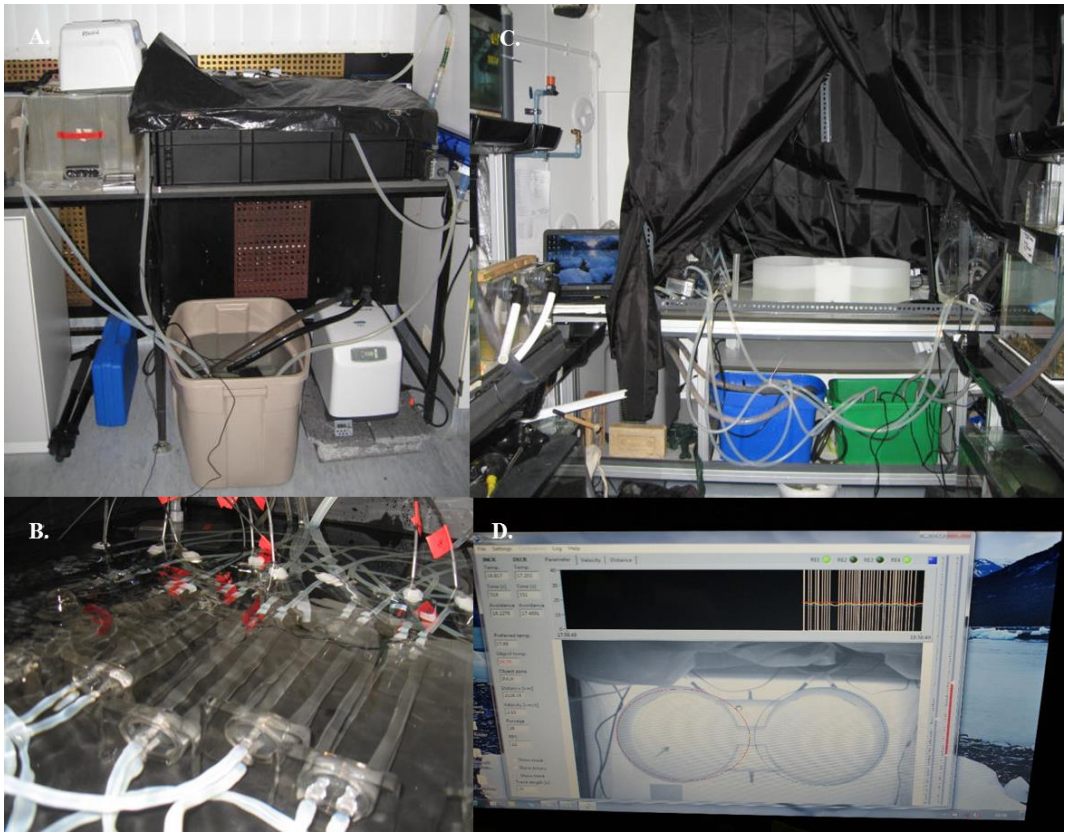
arena. The software detected the position of fish in the arena and regulated the incoming flow from the buffer tanks in order to adjust the temperature within each side. Two settings were available: the “static” and “dynamic” modes. When set to static, temperature difference between the sides is constant and maintains a 2°C differential thermal gradient. In contrast, when switched to dynamic mode, temperature was adjusted and controlled by the location of fish within the arena. The 2°C differential thermal gradient between the cold and warm sides was maintained constant in the “dynamic” mode. Accordingly, if the fish was in the warm side, water temperature in the shuttle-box system increased at a rate of 2°C $h^{-1}$ on both sides (up to a pre-set maximum temperature of 23°C) and if the fish moved to the cold side temperature in the shuttle-box decreased by 2°C $h^{-1}$ (up to a pre-set minimum temperature 1°C). This setting allows an individual fish to regulate the environmental temperature it experienced by moving between two different thermal sides. For each trial, an individual fish was put in the shuttle-box approximately at 18:30 after being starved for 24 h and left undisturbed overnight in the behavioural arena. The shuttle-box was set in static mode whereas the warm side was 1°C warmer and the cold side was 1°C colder than the acclimated temperatures (13°C, 18°C and 21°C) with a water depth of 6 cm (see Photos C., D.). Overnight activity was recorded from the moment at which a fish was introduced in the arena until the next morning (08:00). Overnight activity for each fish was calculated by taking the mean of activity over 8 h (00:00-08:00) and by discarding the first 5 h of recording to avoid activity bias due to handling stress. Morning activity was recorded for one hour (approx. 08:30-09:30). Activity (total distance moved in cm) was calculated by tracking the x-y location coordinates of the fish in the shuttle-box (once every second) using the camera mounted above the behavioural arena, connected to the shuttle-box software. At the end of morning activity measurements, the system was switched to the dynamic mode and fish was left undisturbed for 8 h to select its preferred temperature. The core body temperature was calculated every second by the shuttle-box software using following equation:

$$T_b = T_a + (T_i - T_a) * e^{-kt} \qquad \text{(eq1)}$$

where $T_b$ is core temperature, $T_a$ is ambient water temperature, $T_i$ is the initial temperature of fish, $t$ is time between temperature readings (min), and $k$ is the rate of core temperature varying with body size. Estimates for $k$ were not available for either bullhead or stone loach, and therefore an estimate of $k$ and mass ($m$) from another freshwater teleost fish, the white sucker *Catostomus commersoni,* was used for both species (Stevens & Fry, 1974):

$k=3.69*m^{-0.574}$ (eq2)

The final preferred temperature for each individual fish at each acclimation temperature was calculated by taking the average core body temperature reached during the final 2 h in the shuttle-box of each trial (Killen 2014). At this stage it was assumed that individual fish had reached its preferred thermal range over the whole 8 h trial. After the preferred temperature experiment, fish was returned to initial tank and replaced by another fish in shuttle-box to be tested.




Photos of experimental setups: A. Experimental setup for acute and acclimated respirometry trials, B. Respirometry chambers used during acute and acclimated respirometry trials, C. Experimental setup for preferred temperature and activity level trials, D. SHUTTLESOFT software tracking individual fish the shuttle-box system during preferred temperature trial.

## 4.4 Data and statistical analysis

### 4.4.1 Metabolic rate data

All $MO_2$ data from Firesting $O_2$ software were analysed in LabChart 7 (ADInstruments Pty Ltd, Bella Vista, New South Wales, Australia). To calculate $MO_2$, the average slope of each off flushing measurement period derived from the linear regressions between $O_2$ consumption over time were used. All $MO_2$ data were corrected for the volume of the respirometry chamber and body mass (g) of the fish. Though, bacterial background respiration was considered minimal (less than 30 %) over all respiratory trials, it was taken into account in the calculation of $MO_2$ data; a linear increase in bacterial $MO_2$ consumption over time (before and after trial measurements) was assumed. For graphical representation and to compare $MO_2$ data between bullheads and stone loaches for both thermal trials (acute and acclimated at 13 and 21°C), MMR and SMR were corrected for differences in body mass according to these equations to account for different weight ranges for both species in the two respirometry trials:

For MMR: $MMR_{(i)}*(bm/mean\ bm)^{(1-b)}$ (eq3)

For SMR: $SMR_{(i)}*(bm/mean\ bm)^{(1-b)}$ (eq4)

where $MMR_{(i)}$ and $SMR_{(i)}$ are the initial values (both in mg $O_2$ $kg^{-1}$ $h^{-1}$) for each fish, *bm* is the body mass of each fish, *mean bm* is the calculated average body mass of all fish (*mean bm* = 6.43 g) tested in both respirometry trial (acute and acclimated) and *b* is the scaling exponent (*b* = 0.802 for SMR, Killen et al., 2010 and *b* = 0.803 for MMR, Killen et al., 2016). Aerobic scope (AS, in mg $O_2$ $kg^{-1}$ $h^{-1}$) was calculated as the absolute difference between MMR and SMR. The mass specific $MO_2$ data was only used to compare between bullheads and stone loaches for both thermal trials (acute and acclimated at 13 and 21°C) as different individual fish were used between acute and acclimated thermal trials. The graphs presented in this study used this $MO_2$ data (in mg $O_2$ $kg^{-1}$ $h^{-1}$) (Figure 4.1).

Within each thermal trial, acute or acclimated thermal trial, I used the non-mass specific $MO_2$ data (in mg $O_2$ $h^{-1}$).

For the acute thermal trials, differences in MMR, SMR and AS (mg $O_2$ $h^{-1}$) were tested separately using repeated measures GLM with five temperatures, two fish species and two

cohort groups as categorical variables, body mass of fish (g) as continuous variable and included an error correction term on individual fish measured over temperatures. Within each cohort, the same individual fish were tested at all temperatures (13, 15, 17, 19 and 21°C), therefore I needed to correct for repetition measurements on the same individual fish over temperature. No interaction term between Fish species*temperature was included in this analysis since this interaction was covered by the correction term in GLM. MMR, SMR, AS and mass were log-transformed.

For thermally acclimated trials, differences in MMR, SMR and AS (mg $O_2$ $h^{-1}$) were analysed separately using GLM with temperature, species as a categorical variable, body mass (g) as continuous variable and an interaction term Fish species*temperature. MMR, SMR, AS and mass were log-transformed.

Comparisons for MMR, SMR and AS (mg $O_2$ $kg^{-1}$ $h^{-1}$) between acute and acclimated fish at 13 and 21°C were tested using GLMs with thermal test as a categorical variable (acclimated versus acute) in models for bullheads or stone loaches separately.

### 4.4.2 Activity level

Differences in activity level, overnight and morning activities for bullheads and stone loaches were tested separately by using GLMs with species as categorical variable in the model at each acclimation temperature. GLMs were used to determine the combined effects of variables on the activity levels (overnight and morning tested individually) in bullheads and stone loaches. In each model, species and acclimation temperature were included as categorical covariates, AS (in mg $O_2$ $h^{-1}$), SMR (in mg $O_2$ $h^{-1}$), activity level (overnight or morning activity) and body mass (g) as continuous covariates. Three interaction terms were integrated in the models Fish species*AS, Fish species*SMR and Fish species*acclimation temperature, to test whether the effect metabolic traits on activity levels differ between the two species and to see differences in activity between the two species across acclimation temperatures.

### 4.4.3 Preferred final temperature

Differences in final preferred temperature reached during the last 2 h in the shuttle-box between bullheads and stone loaches were analysed using GLMs with fish species as categorical variable at each acclimation temperature individually. GLM was also used to determine the combined effects of variables on final preferred temperature with

temperature and species as categorical variables, AS (in mg $O_2$ $h^{-1}$), SMR (in mg $O_2$ $h^{-1}$), activity levels (overnight and morning activities in m $h^{-1}$) and body mass (g) as continuous covariates. Three interaction terms were included in the model Fish species*AS, Fish species*SMR and Fish species*acclimation temperature to examine whether the effect of metabolic traits of preferred temperature differs between species and to test for differences in preferred temperature between the two species over the acclimation temperatures.

For the models for activity level and preferred temperature, AS (in mg $O_2$ $h^{-1}$), SMR (in mg $O_2$ $h^{-1}$), body mass (g) and overnight activity (m $h^{-1}$) were log-transformed. Due to zero values, morning activity was log + 1-transformed. For the model for activity level and preferred temperature, non-significant interaction terms were dropped one by one (threshold value $p > 0.5$) and models were re-run followed by post-hoc Tukey multiple comparison procedures. All statistical analyses were performed in R v.3.3.0 and the significance level of all tests was $p = 0.05$.

## 4.5 Results

### 4.5.1 Effect of temperature on metabolic traits during acute thermal trials

For the acute thermal trials, SMR increased with temperature (from 13 to 21°C), irrespective of the direction of the temperature shift, in both bullheads and stone loaches (owrm GLM for temperature effect: SMR, $F_{4,64} = 26.157$, $p < 0.001$; cohort, $F_{1,12} = 0.465$, $p = 0.513$). No differences in SMR were observed between bullheads and stone loaches ($F_{1,12} = 1.739$, $p = 0.220$). Temperature did not have an effect on MMR in either species ($F_{4,64} = 1.328$, $p = 0.274$), though stone loaches had a significantly higher MMR (species effect: $F_{1,12} = 7.060$, $p = 0.026$; Figure 4.1A). Aerobic scope did not increase with temperature in either species ($F_{4,64} = 2.073$, $p = 0.10$). Even though AS seemed to be higher in stone loaches, no significant difference in AS between two fish species could be found ($F_{1,12} = 0.801$, $p = 0.394$; Figure 4.1B). The direction of the temperature shift during trials did not affect estimates of MMR ($F_{1,12} = 1.236$, $p = 0.295$) or AS ($F_{1,12} = 0.603$, $p = 0.457$).

### 4.5.2 Effect of temperature on metabolic traits during acclimated thermal trials

SMR in both species increased with acclimation temperature (GLM, effect temperature: $F_{2,33} = 9.815$, $p < 0.001$). No differences in SMR between bullheads and stone loaches was found across temperatures (fish species: $F_{1,33} = 1.164$, $p = 0.290$). There was no effect of temperature on MMR in both species ($F_{2,33} = 0.967$, $p = 0.393$). Stone loaches displayed a higher MMR than bullheads ($F_{1,33} = 10.354$, $p = 0.003$). Although both fish species showed similar trends in MMR over tested temperature range (fish species*temperature: $F_{2,33} = 0.350$, $p = 0.708$) (Figure 4.1C). Temperature had no effect on aerobic scope in either species ($F_{2,33} = 0.471$, $p = 0.63$). Aerobic scope in stone loaches was higher ($F_{1,33} = 13.671$, $p < 0.001$) but both species displayed no difference in trends for AS over tested temperature range (fish species*temperature: $F_{2,33} = 0.559$, $p = 0.578$; Figure 4.1D).

Comparing SMR, MMR and AS between acute and acclimated fish at 13 and 21°C, bullheads showed no differences in MMR, SMR and AS at 13°C (MMR, GLM, $F_{1,9} = 0.154$, $p = 0.705$; SMR, GLM, $F_{1,9} = 2.618$, $p = 0.144$; AS, GLM, $F_{1,9} = 0.545$, $p = 0.481$; Figure 4.1) and at 21°C (MMR, GLM, $F_{1,10} = 0.09$, $p = 0.771$; SMR, GLM, $F_{1,10} = 0.742$, $p = 0.411$; AS, GLM, $F_{1,10} = 0$, $p = 0.998$; Figure 4.1). No differences for MMR, SMR and AS at 13°C (MMR, GLM, $F_{1,13} = 0.046$, $p = 0.835$; SMR, GLM, $F_{1,13} = 1.237$, $p = 0.288$;

AS, GLM, $F_{1,13} = 0.006$, $p = 0.94$; Figure 4.1) for acute and acclimated stone loaches were found. At 21°C no differences in MMR and AS (MMR, GLM, $F_{1,12} = 0.358$, $p = 0.562$; AS, GLM, $F_{1,12} = 1.161$, $p = 0.304$; Figure 4.1) were found. At 21°C stone loaches had a higher SMR during acute thermal trials compared to acclimated trials by 23.17% (SMR, GLM, $F_{1,12} = 5.217$, $p = 0.043$; Figure 4.1).

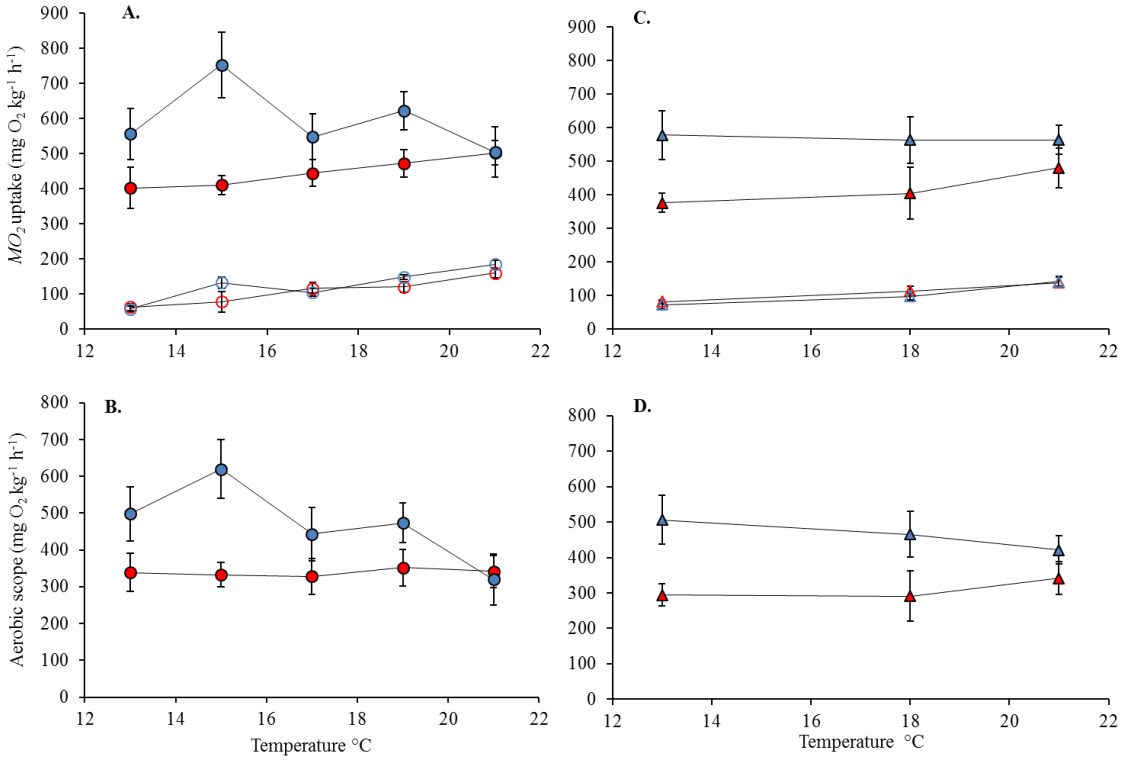



Figure 4.1 Acute thermal exposed (A., B.) oxygen uptake mg $O_2$ $kg^{-1}$ $h^{-1}$ for stone loaches (blue circles) and bullheads (red circles) at 13, 15, 17, 19 and 21°C (mean ± s.e.m.). A. Maximum metabolic rates are represented by filled circles and standard metabolic rates are shown by empty circles. B. Aerobic scope (mg $O_2$ $kg^{-1}$ $h^{-1}$) of stone loaches and bullheads while being acute thermal exposed to 13, 15, 17, 19 and 21°C. Acclimated thermal metabolic phenotype in stone loaches and bullheads (C., D.). C. Maximum (filled triangles) and standard (empty triangle) metabolic rates (C.) in stone loaches and bullheads acclimated to 13, 18 and 21°C. D. Aerobic scope (mg $O_2$ $kg^{-1}$ $h^{-1}$) of stone loaches and bullheads while being acclimated to 13, 18 and 21°C. All $MO_2$ was standardised to mean mass of all fish involved in acclimated and acute thermal trials (m = 6.43 g).

### 4.5.3 Daily activity levels in bullheads and stone loaches

#### Overnight activity

Differences in overnight activity (over 8 h) between bullheads and stone loaches were found at 18°C (GLM, $F_{1,10} = 6.895$, $p = 0.028$; Figure 4.2A) and 21°C (GLM, $F_{1,9} = 18.53$, $p = 0.003$; Figure 4.2A) whereas at the coldest acclimation temperature 13°C, activity level did not differ between species (GLM, $F_{1,10} = 1.614$, $p = 0.236$; Figure 4.2A). In the GLM model to determine the combined effects of variables on overnight activity in bullheads and stone loaches, both interaction terms Fish*logAS and Fish*logSMR were considered in the final model (threshold $p < 0.5$) with Fish*logAS being significant (Figure 4.3A; Table 4.1; G

LM, $F_{1,31} = 9.095$, $p = 0.006$). Stone loaches with high aerobic scope values displayed high er overnight activities. Overnight activity did not increase significantly with temperature (F igure 4.2A; Table 4.1; GLM, $F_{2,31} = 3.217$, $p = 0.06$, all post hoc comparison cases $p > 0.05$ ). Stone loaches were more active than bullheads (Figure 4.2A; Table 4.1; GLM, $F_{1,31} = 18.011$, $p < 0.001$).

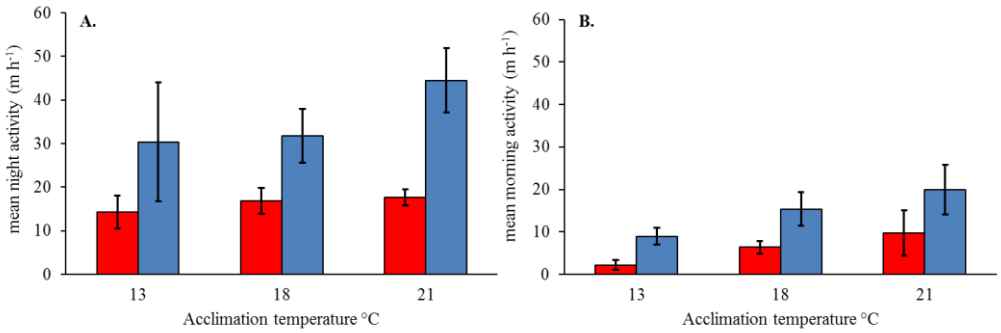



Figure 4.2 Activity levels (mean ± s.e.m.) at different acclimation temperatures (13, 18 and 21°C) in stone loaches (blue bars) and bullheads (red bars) (n = 5-6); A. Mean overnight activity recorded over 8 h; B. Mean morning activity recorded over 1 h.

Table 4.1 General linear model results for the effects on overnight activity (log m $h^{-1}$, 8h) in bullheads and stone loaches acclimated to 13, 18, 21°C, n = 5-6 per temperature treatment (n = 32 in total). Reference for fish species (Fish) "bullhead" and reference acclimation temperature 13°C.

| Term | d.f. | Mean squares | *F* | *p* | Parameters | s.e.m |
|---|---|---|---|---|---|---|
| Stone loach | 1 | 0.666 | 18.011 | <0.001 | 0.115 | 0.239 |
| Acclim temperature (13) | 2 | 0.119 | 3.217 | 0.06 | 0 | 0 |
| (18) | | | | | 0.122 | 0.107 |
| (21) | | | | | 0.246 | 0.129 |
| logAS | 1 | 0.006 | 0.151 | 0.702 | -0.547 | 0.39 |
| logSMR | 1 | 0.020 | 0.534 | 0.473 | -0.251 | 0.441 |
| logmass | 1 | 0.012 | 0.312 | 0.582 | -0.112 | 0.445 |
| logmorn activity | 1 | 0.030 | 0.820 | 0.38 | -0.151 | 0.109 |
| Stone loach*logAS | 1 | 0.336 | 9.095 | 0.006 | 1.137 | 0.516 |
| Stone loach*logSMR | 1 | 0.058 | 1.559 | 0.225 | 0.520 | 0.416 |
| Residuals | 22 | 0.037 | | | | |

*Interaction term Fish*acclimation temperature was included in the original model but removed when not significant.*

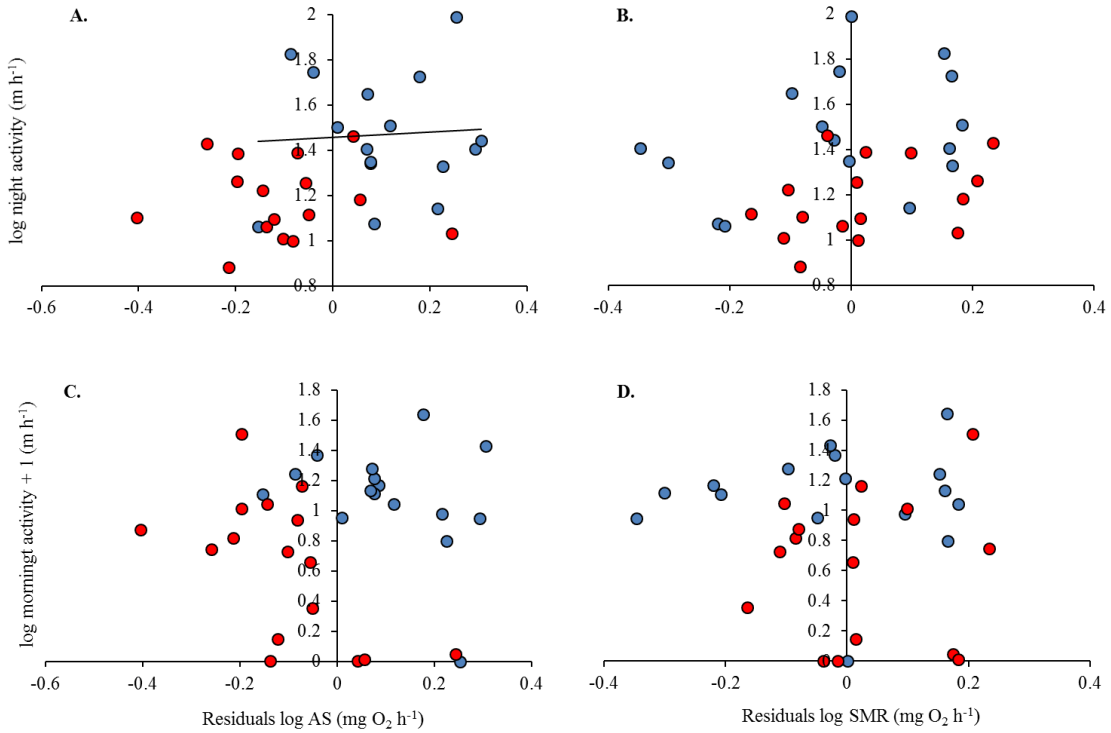



Figure 4.3 Relationship between overnight activity level (log m $h^{-1}$) in individual stone loaches (blue circles) and bullheads (red circles) and metabolic traits (top graphs). A. Residuals aerobic scope (AS) (log mg $O_2$ $h^{-1}$) using regression equation logAS (mg $O_2$ $h^{-1}$) = 0.8007*logmass – 0.2893 all fish included. B. residuals standard metabolic rate (SMR) (log mg $O_2$ $h^{-1}$) using regression equation logSMR (mg $O_2$ $h^{-1}$) = 1.0465 * logmass – 1.0264 all fish included. Relationship between morning activity level (log m $h^{-1}$+1) in individual stone loaches (blue circles) and bullheads (red circles) in metabolic traits (bottom graphs). C. Residual aerobic scope (AS) (log mg $O_2$ $h^{-1}$); D. Residual standard metabolic rate (SMR) (log mg $O_2$ $h^{-1}$). Each point represents one individual. For overnight activity, stone loaches with high AS showed higher activity levels compared to bullheads represented by the solid regression line (see Table 4.1). For morning activity (log m $h^{-1}$ + 1), fish with higher AS (log mg $O_2$ $h^{-1}$) showed lower morning activity. Standard metabolic rate (SMR) and overnight activity did not explain the observed variation in morning activity (see Table 4.2).

## Morning activity

No differences in morning activity between bullheads and stone loaches were found at any experimental temperature (Figure 4.2B; 13°C, GLM, $F_{1,10} = 3.98$, $p = 0.077$; 18°C, GLM, $F_{1,10} = 3.487$, $p = 0.095$; 21°C, GLM, $F_{1,9} = 3.504$, $p = 0.098$). While looking at the combined effects of variables on morning activity in bullheads and stone loaches, interaction terms Fish*logSMR and Fish*acclimation temperature were not included (GLM, $p > 0.5$) in the final model. Morning activity did not increase significantly with temperature in both species (Figure 4.2B, Table 4.2; GLM, $F_{1,31} = 3.339$, $p = 0.053$, all post hoc comparison cases $p > 0.05$) but was higher in stone loaches than bullheads (Figure 4.2B; Table 4.2; GLM, $F_{1,31} = 13.467$, $p = 0.001$). Fish with higher aerobic scope showed reduced morning activity (Figure 4.3C; Table 4.2; GLM, $F_{1,31} = 4.303$, $p = 0.049$). Neither SMR nor overnight activity were relevant in determining morning activity (Figure 4.3, Table 4.2)

Table 4.2 General linear model results for the effects on morning activity (log m $h^{-1}$ + 1, 1 h) in bullheads and stone loaches acclimated to 13, 18, 21°C, n = 5-6 per temperature treatment (n = 32 in total). Reference for fi sh species (Fish) "bullhead" and reference acclimation temperature 13°C.

| Term | d.f. | Mean squares | *F* | *p* | Parameters | s.e.m |
|---|---|---|---|---|---|---|
| Stone loach | 1 | 1.693 | 13.467 | 0.001 | 0.291 | 0.337 |
| Acclim temperature (13) | 2 | 0.42 | 3.339 | 0.053 | 0 | 0 |
| (18) | | | | | 0.426 | 0.181 |
| (21) | | | | | 0.593 | 0.224 |
| logAS | 1 | 0.541 | 4.303 | 0.049 | -1.803 | 0.617 |
| logSMR | 1 | 0.001 | 0.006 | 0.938 | -0.603 | 0.588 |
| logmass | 1 | 0.574 | 4.563 | 0.044 | 1.328 | 0.773 |
| lognight activity | 1 | 0.082 | 0.654 | 0.427 | -0.557 | 0.362 |
| Stone loach*logAS | 1 | 0.384 | 3.050 | 0.094 | 1.665 | 0.954 |
| Residuals | 23 | 0.126 | | | | |

*Interaction terms Fish*acclimation temperature, Fish*logSMR were included in the original model but removed when not significant.*

### 4.5.4 Preferred temperature in bullheads and stone loaches

Preferred temperatures were different between stone loaches and bullheads depending on t he acclimation temperatures (18°C, GLM, $F_{1,10} = 21.72$, $p = 0.001$; 21°C, GLM, $F_{1,9} = 31.3$ 9, $p < 0.001$; Figure 4.4). At 13°C, no significant difference was observed between the two species (GLM, $F_{1,10} = 1.894$, $p = 0.202$; Figure 4.4). Stone loaches preferred warmer tempe ratures (21.07 ± 1.12°C, 22.13 ± 0.27°C, 22.40 ± 0.13°C; Figure 4.4) compared to bullhead s (19.11±0.79°C, 17.48 ± 0.88°C, 17.83 ± 0.80°C; Figure 4.4) at 18 and 21°C. Fish with hi gh SMR tended to have a preference of choosing warmer final preferred temperatures (Fig ure 4.5B, Table 4.3; GLM, effect of logSMR: $F_{1,31} = 5.054$, $p = 0.037$). The other covariate s in the model, acclimation temperature, AS, as well activity levels (Figure 4.5, Table 4.3) were not relevant in determining final preferred temperature.

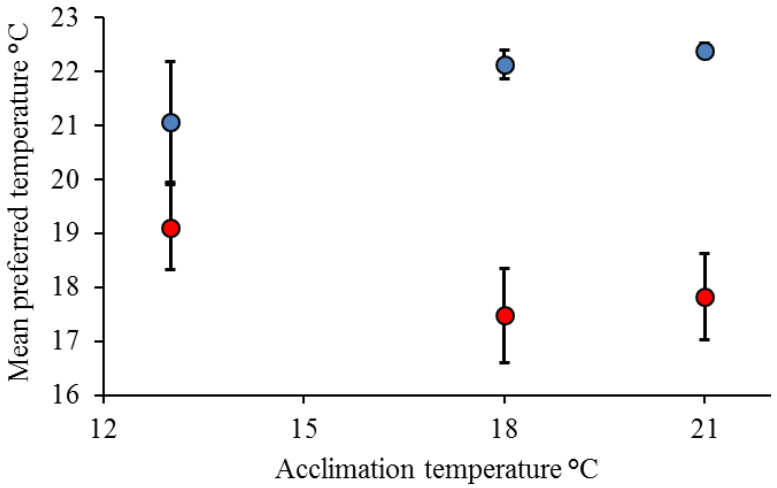



Figure 4.4 Mean preferred temperature (mean ± s.e.m) for stone loaches (blue circles) and bullheads (red circles) (n = 5-6) at three acclimation temperatures (13, 18 and 21°C).

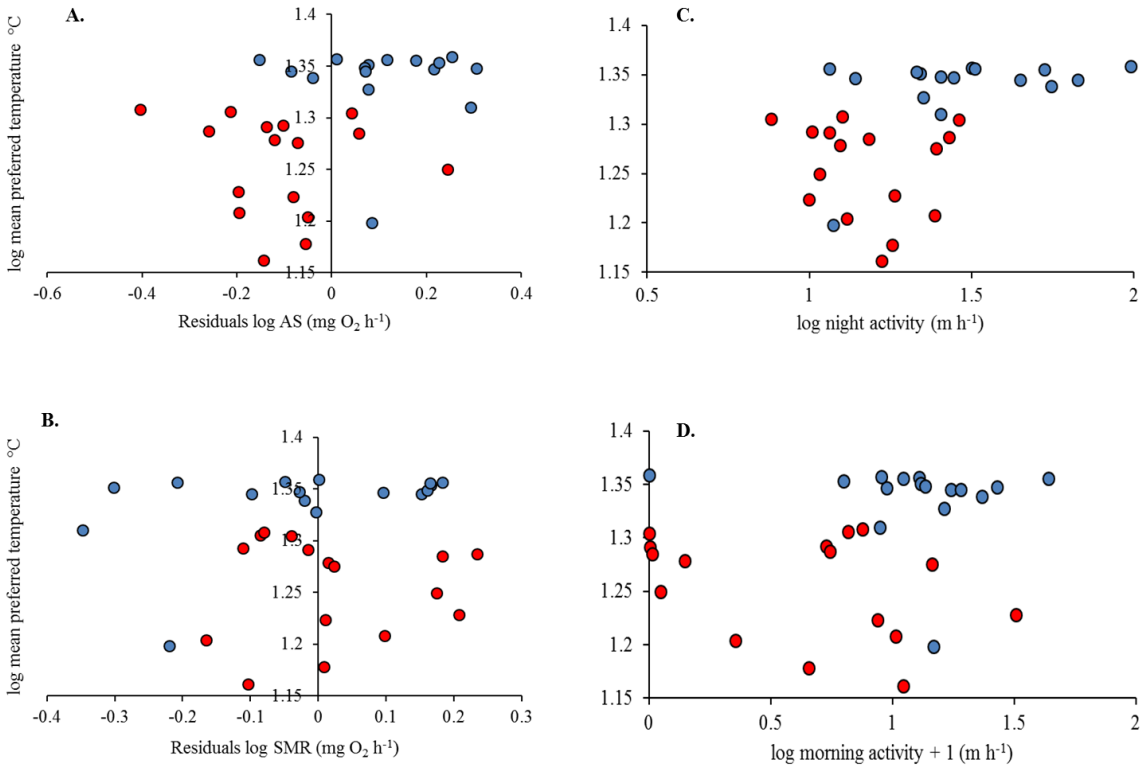



Figure 4.5 Relationship between log transformed mean preferred temperature (°C) in individual stone loaches (blue circles) and bullheads (red circles) and metabolic and behavioural traits. A. Residuals for aerobic scope (AS) (log mg $O_2$ $h^{-1}$) were derived using the regression equation logAS (mg $O_2$ $h^{-1}$) = 0.8007 * (logmass) – 0.2893 all fish included. B. Residuals for standard metabolic rate (SMR) (log mg $O_2$ $h^{-1}$) were derived using the regression equation logSMR (mg $O_2$ $h^{-1}$) = 1.0465 * (logmass) – 1.0264 all fish included. C. Mean night activity level recorded over 8 h; D. Mean morning activity level recorded over 1 h. Each point represents one individual. Fish with high SMR across all temperatures tended to prefer warmer temperatures (see Table 4.3).

Table 4.3 General linear model results for the effects on final preferred temperature in bullheads and stone loaches acclimated to 13, 18, 21°C, n = 5-6 per temperature treatment (n = 32 in total). Reference for Fish species (Fish) "bullhead" and reference acclimation temperature 13°C.

| Term | d.f. | Mean squares | *F* | *p* | Parameters | s.e.m |
|---|---|---|---|---|---|---|
| Stone loach | 1 | 0.054 | 29.865 | <0.001 | -0.025 | 0.076 |
| Acclim temperature (13) | 2 | 0.000 | 0.120 | 0.888 | 0 | 0 |
| (18) | | | | | -0.058 | 0.031 |
| (21) | | | | | -0.064 | 0.038 |
| logAS | 1 | 0.000 | 0.008 | 0.930 | -0.145 | 0.092 |
| logSMR | 1 | 0.009 | 5.054 | 0.037 | 0.221 | 0.105 |
| logmass | 1 | 0.000 | 0.250 | 0.623 | 0.021 | 0.099 |
| log +1 morn activity | 1 | 0.004 | 2.086 | 0.165 | -0.04 | 0.025 |
| lognight activity | 1 | 0.001 | 0.392 | 0.539 | 0.023 | 0.047 |
| Stone loach*logAS | 1 | 0.000 | 0.031 | 0.862 | 0.107 | 0.130 |
| Stone loach*logSMR | 1 | 0.000 | 0.213 | 0.65 | -0.200 | 0.127 |
| Stone loach*13°C | 2 | 0.004 | 2.336 | 0.124 | 0 | 0 |
| Stone loach*18°C | | | | | 0.086 | 0.042 |
| Stone loach*21°C | | | | | 0.093 | 0.052 |
| Residuals | 19 | 0.002 | | | | |

## 4.6 Discussion

### 4.6.1 Differences in metabolic responses over temperature changes

The results of this study suggest that stone loaches and bullheads show little difference in their metabolic response to either acute or long term variation in temperatures. For example, both species showed similar increases in SMR with temperature during both the acute or acclimated trials. In addition to similar slopes in SMR with temperature, the elevation for SMR was also comparable between the two species. Previous studies have found lower SMR values in invasive amphipod species compared to native species and it was suggested to have contributed the invasion success of this species (Maazouzi et al., 2011; Becker et al., 2016). During unfavourable conditions (e.g. hypoxic events) a lower SMR could translate into a greater ability to survive periods of limited and unpredictable food availability due to reduced maintenance requirements (Reid et al., 2012). This was unlikely to be a mechanism allowing bullheads to outcompete stone loaches given that the two species showed similar values for SMR over a range of temperatures.

The effect of temperature on metabolic traits (SMR, MMR and AS) in both species were similar in both the acute and acclimated trials. Over acute thermal trials, stone loaches' MMR and AS tended to increase with temperatures, peaked at 15°C and decrease with further warming. MMR in bullheads increased with temperature until 21°C and AS stayed consistent across all temperatures. However, these changes in metabolic rates over acute temperature changes were not statistically significant. Still, AS in bullheads seemed to be less affected by acute temperature changes, possibly reflecting an ability to maintain their capacity for aerobic metabolism over thermal fluctuations in riverine systems. Nevertheless, both species were able to maintain AS over same thermal range (13-21°C).

However, some interesting trends emerged when comparing the acute and acclimated trials within each species at 13°C and 21°C. Notably, stone loaches showed a 23% reduction in SMR at 21°C during acclimated trials as compared to acute trials, indicating that this species is capable of a certain degree of thermal compensation with acclimation in response to warming. This can be achieved by adjusting membrane structure and fluidity as well the function or density of aerobic enzymes (Seebacher et al., 2010). In bullheads no compensatory responses were observed at 13 nor 21°C, with no differences in any of AS, MMR and SMR between the acute and acclimated thermal trials. Previous studies have demonstrated differences in thermal performance breadths and optima for aerobic metabolism between invasive and native amphipod species, where the invasive species optimised its aerobic metabolism at colder temperatures (5-10°C) compared to native species (10-20°C) (Maazouzi et al., 2011; Becker et al., 2016). In another example, invasive marbled spinefoot (*Siganus rivulatus*) had an optimal temperature for AS at a warmer temperature (29.1°C) than native salema (*Sarpa salpa*; 21.8°C) which could potentially give this invasive species an advantage in warming climates (Marras et al., 2015). These mechanisms are unlikely to have facilitated the expansion of bullheads into the range of stone loaches given that these two species didn't differ in their thermal ranges for aerobic scope (Figure 4.1).

Although, differences in MMR and AS were observed between the two species with stone loaches having higher MMR and AS than bullheads in acute or acclimated trials. These differences in MMR and AS might be related to differences in foraging behaviour and swimming capacities between the two fish species. Stone loaches are more active (Tudorache et al., 2008) foragers, searching for food in the substrate with their sensory tactile barbels (Smyly 1955) during night time (Fischer 2004). Bullheads, in contrast,

despite living in the same environment as stone loaches, have less active lifestyle, mainly hiding under rocks and stones and ambushing prey (Smyly 1957; Tomlinson & Perrow 2003). Indeed, in the current study, I observed differences in night and morning activity between bullheads and stone loaches, with stone loaches being generally more active than bullheads. Both species were more active during the night as compared to the morning, agreeing with previous observations of these species (Smyly 1955, 1957; Fischer 2004; Nunn et al., 2010). There was also a general positive effect of temperature on activity levels (overnight and morning) even though the effect was not statistically significant (Figure 4.2). Conversely, stone loaches had higher AS compared to bullheads even though the difference was not statistically significant, may therefore have an advantage over bullheads in competitive interactions, as they can allocate more energy into agonistic behaviour.

### 4.6.2 Differences in preferred temperatures

For temperature preference, stone loaches chose warmer temperatures compared to bullheads at each of the acclimation temperatures. Temperature preference choice was independent of the acclimation temperature. These findings coincided with results obtained in previous study in eurythermal fish species (Khan & Herbert 2012). However, stone loaches preferred temperatures (21.4-22°C) above the tested thermal range for aerobic scope (13-21°C). It is notable that stone loaches were the most active at 21°C. By choosing warmer temperatures, stone loaches might gain a thermodynamic advantage in biochemical processes (Angilletta et al., 2010) which they may use to achieve increased activity levels. Due to different preferred temperature ranges observed between these two species, they may choose different thermal habitats if given the choice. Additionally, with rising temperatures, invasive bullheads might be less favoured as the native stone loaches.

### 4.6.3 Links between metabolic traits and activity levels

A positive link between aerobic scope (AS) and night activity was found in stone loaches, while in bullheads no relationship was found between night activity level and metabolic traits. For morning activity, a reverse pattern was observed, in both species individuals with a higher AS showed reduced activity. Both species are nocturnal and are mostly inactive during the daytime. Therefore, they might reduce energy expenditure to activity and prioritise other oxygen demanding processes during the day. However, as bullheads have already low baseline activity levels, less energy is invested in activity, and therefore

more energy may be available to allocate and contribute to other energy-demanding processes (e.g. growth, reproduction and immune system).

### 4.6.4 Links between metabolic traits and preferred temperatures

There was a positive effect of standard metabolic rate (SMR) on thermal preference in both fish species. Individuals with high SMR across all acclimation temperatures, showed a tendency to choose warmer final preferred temperatures (Figure 4.5B). On the contrary, Killen (2014) found that minnows *Phoxinus phoxinus* with a higher SMR at 14°C tended to choose colder temperatures. In that study, minnows chose colder temperatures to presumably reduce maintenance costs which enable them to reallocate surplus energy to growth. In bullheads and stone loaches, choosing warmer temperature might provide a thermodynamic advantage in biochemical processes such that individuals with higher maintenance requirements can achieve higher rates of foraging. While this rationale may apply to the actively foraging stone loaches, it remains a mystery why the mainly ambush foraging bullheads with a high SMR would also select warmer temperatures. More research is needed to understand the interactions among acclimation temperature, foraging behaviour, growth rates, and reproductive output in this species.

Among individual fish, no correlation was found between baseline activity and choice of preferred temperature across all acclimation temperatures. These findings were in contradiction to the study on minnows by Killen (2014). More active minnows tended to choose warmer temperatures. Fish thermoregulate by moving or migrating to different habitats (Freitas et al., 2015). The frequency and magnitude of these thermoregulating movements depend on the swimming and locomotive capacity of the fish species, the gradient of thermal differences within the environment, and the biological necessity to thermoregulate. Here, both fish species are benthic species with limited swimming capacities are unable to undergo substantial migrations or thermoregulating movements (Tudoarche et al., 2008; Nunn et al., 2010). The migration range recorded for bullheads was 133 meters (mean value during spawning season, Knaepkens et al., 2004). In summary, among individuals, temperature preference in bullheads and stone loaches appears to be more related to SMR and is not their intrinsic propensity toward activity at a given acclimation temperature.

The motivation to explore in both species, therefore the desire to decrease, increase or shift their daily activity patterns might change depending on food availability, predator presence, seasons and population of interest. Stone loaches did not increase their daytime activity even under highly food withdrawal cases (without food over 22 days) and remained more active during night time (Fischer 2004). In the presence of predator, daytime activity ceased completely in stone loaches (Fisher 2004). This indicates that the motivation to increase daytime activity is not driven by food deprivation in stone loaches even with no acute predator threat. The food withdrawal of 24 h used in this study was surely not affecting the natural activity patterns in stone loaches. As for bullheads, it has been shown that during the spawning season (May-June), more mobile male individual bullheads increased their distance travelled to find suitable spawning sites and build nests (Knaepkens et al., 2004). In this case, the motivation of an increase of activity levels was set by the reproduction season and was more pronounced in males. Here, in my study I did not identify the sex of individual fish involved in the trials and the experiments were performed over March-April reflecting the start of the reproduction season in bullheads. No reproduction or spawning was observed in both species over the period of the experiments. Furthermore as the experimental fish were exposed to constant photoperiod of a 12:12 light:night period, it was unlikely that the activity levels were affected by reproductive behaviour in bullheads nor in stone loaches.

### 4.6.5 Conclusions

Both fish species showed similar metabolic responses over acute and acclimated temperature changes giving an explication to their observed co-occurrence in streams. Despite this, differences in metabolic rates between both species have been observed. Bullheads had lower MMR and AS values. Differences in activity levels and preferred temperatures were found between these two species. Bullheads were less active and preferred colder temperatures. Bullheads are highly territorial fish and engage frequently into agonistic behaviours with conspecifics and other benthic fish species. With low routine activity levels, bullheads might have more flexibility to allocate the energy in other performance traits such as territorial competition. Although with low activity levels, bullheads may encounter difficulties to spread actively and expand their range. The invasion success of bullheads in Scottish freshwater streams might not be the result of difference in metabolic responses towards temperature changes nor temperature preferences.

This study examined metabolic and behavioural responses to thermal variation to determine whether differential responses to temperature might allow bullheads to be favoured within a given habitat over the native stone loaches. In this study, I focused on the aerobic part of the metabolism in both species. It could be plausible that bullheads may rely more on anaerobic pathways while facing environmental stressors. Further research in comparative invasive ecology needs to be considered such as looking at different organisation levels in the aerobic and anaerobic metabolism (cellular e.g. mitochrondria; molecular, enzyme and organs, e.g. heart and liver) that might be relevant in the invasion success. Additionally, here in this study, temperature was not the abiotic factor facilitating or contributing to the invasion success of bullheads. Other abiotic abiotic factors (e.g. hypoxia) might drive and mitigate interspecific difference in metabolism and behaviour that could contribute to invasion success.

# Chapter 5: The effect of thermal tolerance on competitive outcomes between an invasive and native freshwater fish species

Currently in preparation for submission to Freshwater Biology or Biological Invasions

## 5.1 Abstract

Interspecific competition between invasive and native fish species can change the structure and dynamics of native fish community. To understand such effects, it is necessary to examine the competitive interactions between native and invasive species. Furthermore, these interactions are expected to vary over competitive context (for prey or cover), differ over a range of environmental factors (e.g. temperature) and be linked directly or indirectly to species' metabolic capacity (e.g. aerobic scope). With current environmental changes, the invasive species might gain a competitive advantage over the native species though shifts or changes in competitive behaviour and metabolic ability. Still up to date, it is unclear what underlying mechanisms triggers the invasion success of an invasive species over the native species. Here I compared the competitive ability of two freshwater fish species, bullhead (*Cottus gobio*), an invasive species, and native stone loach (*Barbatula barbatula*), using a single shelter at three different temperatures (13°C, 17°C and 21°C) as the resource competed for. Both fish species were tested twice for shelter use, once individually (one bullhead or one stone loach at the time) to get the baseline cover use and once in competition with a size-matched competitor (one bullhead versus one stone loach) at the different acclimation temperatures. After the competition trials, metabolic rates data (SMR, MMR and AS) were collected at all temperatures for individual fish involved in competition trials to investigate the possible association between intra- and interspecific competitiveness and metabolic capacity. The results showed that, overall, native stone loach was a better competitor for shelter use, in particular at 13°C. At 21°C, both fish species' competitive behaviour was limited. However, despite the difference between the species, no links between competitive behaviour and metabolic capacity were found between species or individuals. As these species are nevertheless known to prefer avoiding each other in the wild, it is possible that the intensity of their competitive interactions is density-dependent and that the density used in the experiments was not high enough to elicit strong competition. In conclusion, while examining competitive interactions between an invasive and native species, it is important to consider the context of the competition.

## 5.2 Introduction

Competition for resources among species is widespread and can have important consequences for species' vulnerability to predation, reproductive success, and geographical distributions (Miller 1967; Fincke 1992; de Gelder et al., 2016; Williams et al., 2016). The outcome of interspecific competition is context-dependent and may vary over different environmental stressors mediating and changing the competitive behaviour (Chamberlais et al., 2014). Depending on the environmental conditions, one species may be favoured over others and but lose this advantage if environmental conditions shift. Context-specific competition (Taniguichi et al., 1998; McHugh & Budy 2005; Oyugi et al., 2012; Carmona-Catot et al., 2013) has been studied in range of different taxa and in response to different environmental factors such as pH levels and salinity (Warner et al., 1993; Costanzo et al., 2005; Alcaraz et al., 2008). With rising temperatures, an increase in invasion success of non-native species is predicted (Rahel & Olden 2008, Britton et al., 2010). Through changes in thermal conditions, non-native species are expected to shift ranges into novel thermally suitable habitats (Sharma et al., 2007) and potentially compete with native species. To date at least 123 aquatic species have been introduced into European countries, with freshwater fish being the most frequently introduced aquatic taxa (Garcìa-Berthou et al., 2005). Interspecific competition can occur during introduction of non-native species that might engage in competitive behaviour with native species for resources such as the availability for food or shelter. Indeed, the outcome of these competitive interactions will determine whether a non-native species will succeed in colonising a novel environment. Although invasion success is believed to increase with temperature (Lehtonen 1996; Rahel et al., 2008), little is known about specific behavioural and physiological traits affect the outcome of competitive interactions between invasive and native species and the degree to which environmental factors modulate these outcomes.

It has been suggested that competitive ability is linked to whole-animal metabolic traits (Seth et al., 2013; Killen et al., 2014). For example, individuals with higher standard metabolic rate (SMR; the minimum energy required for maintenance functions) are more aggressive and dominant (Metcalfe et al., 1995; Cutts et al., 1998). However, aggressive behaviours can include intense physical activity (Seebacher et al., 2013) and can be very costly energetically. Other traits, such as aerobic scope (AS) – which represents an animal's ability to increase its level of aerobic metabolism to fuel physiological processes

above those required for maintenance – may also be relevant for determining which individuals or species have the greatest competitive ability. Those with an increased AS may have the capacity to engage in agonistic behaviours while still carrying out other physiological tasks such as growth or digestion. While many studies have focused on intraspecific links between SMR and dominance (see for details Metcalfe et al., 2016a), very few have examined links between AS and competitive behaviour at either the inter- or intraspecific levels. Killen et al. (2014) found a positive correlation between AS and aggressiveness in juvenile Ambon damselfish during intraspecific competition for territory. Seth et al. (2013) showed a positive link between AS and interspecific competition for shelter among three sculpin species from different geographical regions and with varying thermal optima for AS. Additional information on how physiological traits such as AS influence competitive behaviours would be useful for understanding a variety of ecological phenomena including interactions between native and invasive species (Mills et al., 2004; Blanchet et al., 2007; Kakareko et al., 2014).

Environmental temperature is a major factor influencing both inter- and intraspecific competitions (Carmona-Catot et al., 2013; Amarasekare & Coutinho 2014), probably due to the profound temperature effects on physiological processes, particularly in ectotherms (Brown et al., 2004). AS is known to vary with temperature in ectotherms, generally increasing with temperature until an optimum and then declining with further temperature increases (Farrell 2016). If temperatures shift toward a species' optimal thermal range for AS, more energy may be allocated to competitive behaviour. In the context of invasion success, an invasive species may be favoured with rising temperatures and become more aggressive (Carmona-Catot et al., 2013) which could be related to a higher AS capacity at those temperatures. Various studies have looked at the effect of temperature on competitive outcomes in fish (Taniguichi et al., 1998; McHugh & Budy 2005; Oyugi et al., 2012; Carmona-Catot et al., 2013), however, the complexity of intra-interspecific interactions and variation in reaction norms of traits with temperature makes it difficult to draw general conclusions on the competitive outcomes. Understanding the direct effects of temperature on competitive outcomes between invasive and native species and how physiological traits are shaping these interactions, is crucial for predicting future changes in community structure with further warming.

In this study, I examined competitive interactions for shelter between invasive bullheads (*Cottus gobio*) and native stone loaches (*Barbatula barbatula*) at three different temperatures (13°C, 17°C and 21°C) over the natural thermal range of these species during

the summer period in Scottish rivers. The two species are both bottom-dwelling benthic fish species which use shelter for cover and protection from predation. Stone loaches and bullheads are known to co-exist over their native range (Wheeler 1969) sharing the same ecological niche and are potentially competing for resources such as shelter opportunity (Prenda et al., 1997a). The main questions in this study were; 1) Is competitive ability between bullheads and stone loaches linked to interspecific differences in aerobic scope? 2) Is competitive ability related to aerobic scope within species?; and 3) Does temperature modulate competitive ability or links between competitive ability and aerobic scope? I hypothesised that the invasive bullheads might have higher competitive capacity for shelter over all temperatures tested, and that this might be linked to aerobic scope.

## 5.3 Methods and Materials

### 5.3.1 Experimental Animals

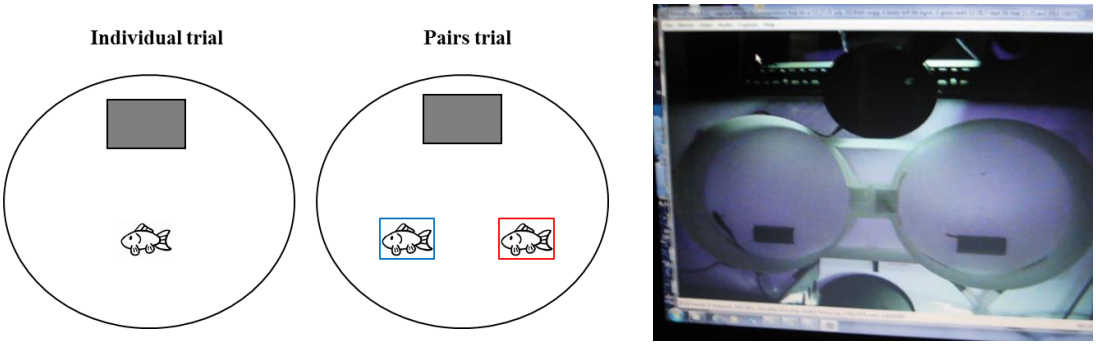
Fish were caught in two different rivers in Scotland, United Kingdom. Stone loaches (*Barbatula barbatula* Linnaeus 1758) were obtained from Rotten Calder Water and White Cart catchment near Pollock. Bullheads (*Cottus gobio* Linnaeus 1758) were caught from the White Cart catchment and transported to Institute of Biodiversity, Animal Health and Comparative Medicine, Glasgow. In the laboratory aquaria fish were held in 92 L (L = 620 mm, W = 620 mm and D = 240 mm) tanks which were supplied with a continuous flow of dechlorinated, UV-sterilised freshwater water at 13-14°C. The photoperiod was a 12:12 light:dark cycle. Fish were fed daily *ad libitum* with frozen bloodworms to satiation and once a week with chunks of small fish. Three months before experiments, all fish (45 stone loaches and 43 bullheads) were tagged for identification using elastomer and allocated among several holding tanks 5 or 6 stone loaches per tank (47 L, L = 520 mm, W = 380 mm and D = 240 mm, total = 6 tanks) 6-7 bullheads per tank (47 L, L = 520 mm, W = 380 mm and D = 240 mm or 30 L, L = 380 mm, W = 380 mm and D = 210 mm, total = 6 tanks). Each of the holding tanks contained gravel substrate, plastic plants and circular plastic pipes to provide shelter.

### 5.3.2 Competition trials

Fish were randomly allocated to three different acclimation temperatures (13°C, 17°C and 21°C ± 0.5°C, 19-21 bullheads and 16-18 stone loaches per temperature) and left undisturbed for two weeks. Temperatures were recorded on a daily basis. After two weeks of acclimation, fish were tested for competitive capacity for shelter use at three acclimation

temperatures in two separated circular arenas (30 cm diameter) run in parallel. To control the temperature within each arena, buffer tanks were installed externally to the system and connected to heating and cooling reservoirs. Within each behavioural arena, temperature was continuously measured with an external temperature probe. Those probes were connected to a computer-driven temperature controller and data acquisition system (DAQ-M; Loligo Systems). The control of temperature within each behavioural arena was maintained by software (SHUTTLESOFT; Loligo Systems) to acclimation temperature tested (± 0.2°C). To test competitive capacity for shelter use, one single fixed shelter (L = 8 cm, H = 2.2-2.3 cm, D = 3.5 cm) was glued in each of the two behavioural arenas between the inflow and outflow channels (~1.5 cm from the walls of the arena). The slow circular movement of water created by the inflow and outflow ports was not disturbed by the shelter and thermal homogeneity within each behavioural arena was maintained. Before each trial, fish were starved for 48 h and fed with bloodworms after the behavioural experiments were finished. Each fish was tested twice, once individually to get a baseline measure of shelter use and once in size-matched pairs (max = ± 3cm) with one bullhead (8.37 ± 0.10 cm; 10.19 ± 0.37 g) and one stone loach (9.79 ± 0.13 cm; 7.23 ± 0.28 g) to assess the competitive ability. Some fish that were acclimated to the different temperatures were not used during competition trials because they were not able to be appropriately size-matched with an individual from the alternate species. Between each competitive trial (individual and pairs), fish were left undisturbed for 24 h before being tested again. The order in which temperature (13°C, 17°C and 21°C) and competition treatments (individual and competition) were performed was randomised over the experimental period. Fish were introduced to the behavioural arena and left undisturbed for 1 h to settle. Each trial lasted 12 min and was recorded by using a webcam placed over the experimental arena. Eight trials were performed per day (two arenas were used and run in parallel) and they lasted for two weeks. 68 videos were collected from the competition trials and were analysed using Solomon Coder (http://solomoncoder.com/). Time spent in shelter (s, in seconds) for each fish was quantified in both the paired and individual trials. Competitive ability was corrected for baseline tendency to use shelter by calculating the difference in time spent in the shelter in paired competitive trials and time spent in the shelter in individual trials (Δtime spent in shelter (pairs - individual trials)). Due to this calculation species had a positive, negative or null scores for the time spent in shelter. Fish displaying a negative score for Δtime spent in shelter showed reduced shelter use under competition compared to when they were in the arena alone. Correspondingly, fish with a positive score had a higher shelter use when in a competitive environment as compared to when they were alone. A

null score would indicate no change in time spent in the shelter across the paired and individual treatments (see below photos of experimental setup).




Photos of experimental setup for competition trials for shelter resource. On the left side, the scheme of the individual trial (one bullhead or one stone loach at the time) and pairs trial (one bullhead (red fish) versus one stone loach (blue fish)) introduced in the behavioural arena to measure the individual use for shelter as a resource and competitive outcome for shelter between bullheads and stone loaches for shelter. On the right, a photo of the actual behavioural arena for the competition arena.

### 5.3.3 Respirometry setup and protocol acclimated thermal trial

After the competition trials, oxygen consumption rates ($MO_2$) of fish were measured using intermittent-flow respirometry system following practices described by Steffensen (1989) and Clark et al. (2013). Four glass respirometers (163 ml total volume) were submerged in a black experimental tank filled with 93 L (L = 780 mm, W = 570 mm, D = 210 mm) with air-saturated water. Temperature within the experimental tank was controlled by a thermostatic reservoir connected to the experimental tank by a thermoregulator (TMP-REG system, Loligo Systems, Tjele, Denmark). To avoid an oxygen gradient in the respirometry chambers, a continuous mixing circuit (100 ml/min) was induced by a peristaltic pump (Masterflex L/S 100 rmp, Cole-Parmer, UK). Two Eheim flush pumps connected to automated digital recycling timer (Superpro Hydroponics MFRT-1) flushed the chambers for 3 min every 13 min with surrounding oxygenated water from the experimental tank. A UV-steriliser was connected to the experimental tank to minimise bacterial respiration during the trials. Oxygen concentrations in chambers was measured every two seconds using a Firesting 4 channel oxygen meter and associated sensors (PyroScience GmbH, Aachen, Germany) (see Photos in Chapter 4 for more details on experimental setup). During the off cycle of 10 min, oxygen respiration rates ($MO_2$) were measured by the decline in oxygen concentration in the respirometry chambers. During the trials, the experimental tank was covered with a black plastic shield to avoid external disturbances. Additionally, the sides of the respirometry chambers were covered with opaque material so the fish were not in visual contact.

After the competition trials, 8-19 bullheads (10.13 ± 0.38 g) and 14-16 stone loaches (7.13 ± 0.26 g) at each acclimation temperature were tested for metabolic rate after being acclimated to three temperatures (13°C, 17°C and 21°C ± 0.5°C) for two weeks. Fish were starved for 48 h before respirometry trials. To obtain maximum metabolic rate (MMR in mg $O_2$ $h^{-1}$) fish were transferred individually in a circular tank (D = 50 cm) with a water depth of 10 cm and manually chased until exhaustion (Killen et al., 2012; Clark et al., 2013). Immediately after completed exhaustion (time of chase: around 1 min 30 s and 3 min), fish were transferred in respirometer chambers. After the chase protocol, the first 5 min of oxygen consumption was used to calculate MMR (in mg $O_2$ $h^{-1}$). Fish were left in the respirometry chambers overnight (22 h undisturbed) to obtain standard metabolic rate at rest. The whole animal standard metabolic rate (SMR, in mg $O_2$ $h^{-1}$) was taken as the 10% percentile values during the measurement period (Killen et al. 2012; Killen 2014) excluding the first 5 h of measurements when oxygen uptake was still elevated due to handling stress and recovery from exercise (Killen 2014). This protocol was repeated for all test temperatures.

### 5.3.4 Data analysis and statistics

For metabolic rate trials, all $MO_2$ data were imported as text files obtained from the Firesting $O_2$ software and analysed in LabChart 7 Pro (ADInstuments Pty Ltd, Bella Vista, NSM, Australia). $MO_2$ (mg $O_2$ $h^{-1}$) data were corrected for the volume of respirometry chamber and volume of tubing in the closed-up system. $MO_2$ measurements were corrected for background bacterial respiration (less than 21 %) by assuming a linear increase in the background respiration over the time of measurements. Aerobic scope was calculated as the difference of MMR-SMR (in mg $O_2$ $h^{-1}$).

Statistical analyses were performed in R statistical software (version 3.3.0 R Foundation for Statistical Computing). The effect of temperature on the competitive outcome (Δtime spent in shelter (pairs - individual trials) in sec) between the two species was analysed using a general linear model (GLM) with temperature treatments (categorical variable with three levels, 13, 17 and 21°C), body mass (continuous variable), body size (continuous variable), species (categorical variable with two levels, bullhead and stone loach) and two two-way interaction terms species*temperature and body mass*body size as explanatory variables in the model. The effects of AS or SMR on competitive ability were assessed between species using GLM with temperature treatments, body mass (continuous variable), species (categorical variable), aerobic scope (AS, in mg $O_2$ $h^{-1}$) or (SMR, in mg $O_2$ $h^{-1}$),

together with an interaction term species*AS or species*SMR in the models. Non-significant interaction terms were dropped one by one (threshold value $p > 0.5$) and models were re-run.

The effect of the three acclimation temperatures (13°C, 17°C and 21°C) on each SMR, MMR or AS (in mg $O_2$ $h^{-1}$) (dependent variable) in bullheads or stone loaches were tested first using GLM with temperature treatments and body mass as explanatory variables in the models followed up with Tukey post hoc multiple comparison tests between temperature treatments. To compare differences in each SMR, MMR or AS (in mg $O_2$ $h^{-1}$) (dependent variable) between bullheads and stone loaches over the tested temperatures, GLM with explanatory variables as temperature treatments, species and body mass were performed. Body mass, body size, AS, MMR and SMR values were log transformed due a non-linear relationship between metabolic rates and body mass. The level of significance was set at $p = 0.05$. Data are represented in figures as means ± s.e.m values unless otherwise indicated.

## 5.4 Results

### 5.4.1 Interspecific differences in competitive ability over different temperatures

Data on competitive ability were obtained for 42 bullheads (13°C n = 15, 17°C n = 15 and 21°C n = 12, 10.19 ± 0.37g, 8.37 ± 0.10 cm) and 42 stone loaches (13°C n = 15, 17°C n = 15 and 21°C n = 12, 7.23 ± 0.28 g, 9.79 ± 0.13 cm). In this study, stone loaches showed a higher competitive ability than bullheads at two extreme temperature treatments 13°C and 21°C, with highest score at 13°C. At 17°C, both fish species displayed increased shelter use while in a competitive environment compared to when they were alone, with neither species having a clear advantage. At 21°C, the tendency to use shelter seemed to decrease in both species (Figure 5.1). Although, no statistical significance was found for the higher competitive ability in stone loaches. Nevertheless, temperature did not have a significant effect on the competitive outcomes between the two species (Table 5.1).

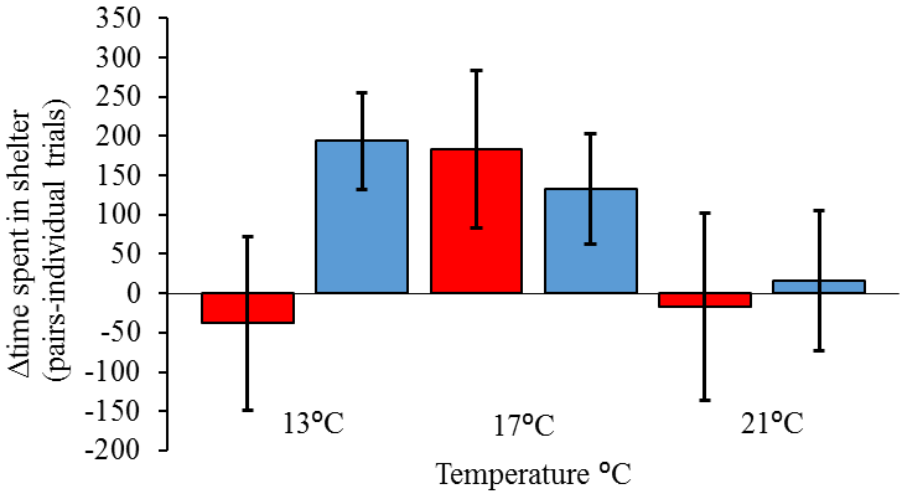



Figure 5.1 The effect of temperature (13°C,17°C and 21°C) on the competitive behaviour (Δtime spent in shelter (pairs - individual trials)) in bullheads (red bars) and stone loaches (blue bars) represented in means ± s.e.m.

Table 5.1 Model (GLM) summary for the effect of temperature on the competitive capacity in bullheads and stone loaches. Explanatory variables estimates (Parameters) and standard error (s.e.m). Reference species was bullhead and reference temperature was 13°C (n = 84).

| **Term** | **d.f.** | **Mean squares** | ***F*** | ***p*** | **Parameters** | **s.e.m** |
|---|---|---|---|---|---|---|
| Acclim temperature (13) | 2 | 168545 | 1.379 | 0.258 | | |
| (17) | | | | | 214.98 | 127.92 |
| (21) | | | | | 35.09 | 139.54 |
| logmass | 1 | 42053 | 0.344 | 0.559 | 630.41 | 727.53 |
| logsize | 1 | 7963 | 0.065 | 0.799 | -2190.22 | 2141.63 |
| Stone loach | 1 | 253962 | 2.078 | 0.154 | 460.90 | 264.41 |
| Stone loach*13°C | 2 | 127055 | 1.039 | 0.359 | | |
| Stone loach*17°C | | | | | -257.29 | 182.29 |
| Stone loach*21°C | | | | | -179.68 | 192.45 |
| Residuals | 76 | 122239 | | | | |

*Interaction terms logmass*logsize was included in the original model but removed when not significant.*

### 5.4.2 Links between metabolic traits and competitive capacity between and within species over different temperatures

From 42 bullheads in competition trials, metabolic traits were measured for 34 bullheads (13°C n = 15, 17°C n = 12 and 21°C n = 7, 10.36 ± 0.417 g, 8.45 ± 0.114 cm) and from 42 stone loaches metabolic rates were collected for 40 stone loaches as one individual died at 17°C and another one at 21°C during the trials (13°C n = 15, 17°C n = 14 and 21°C n = 11, 7.27 ± 0.288 g, 9.82 ± 0.128 cm). Neither AS (Table 5.2A) nor SMR were associated with competitive ability for shelter use (Figure 5.2; Table 5.2B). Furthermore, other morphological features, such as mass, also did not predict competitive outcomes (Table 5.2).

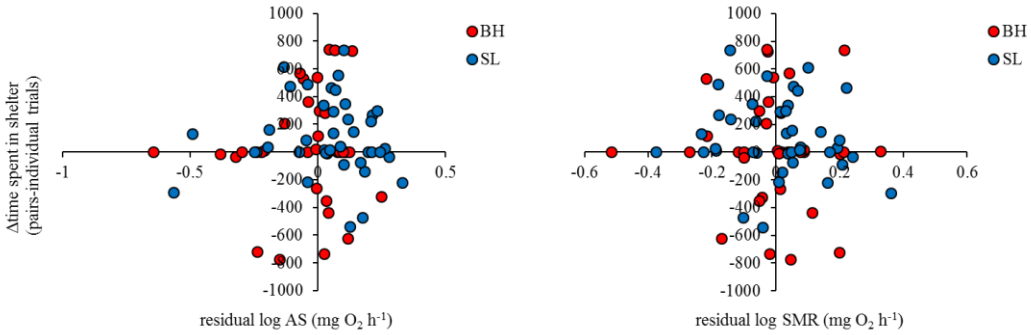



Figure 5.2 The effect of aerobic scope (AS, log mg $O_2$ $h^{-1}$, left) and standard metabolic rate (SMR, log mg $O_2$ $h^{-1}$, right) on the competitive capacity (Δtime spent in shelter (pairs - individual trials)) in bullheads (red dots) and stone loaches (blue dots) across all temperatures. To correct for body mass, I used residuals of logAS (mg $O_2$ $h^{-1}$) = 0.7197 * (logmass) - 0.216 (aerobic scope, log mg $O_2$ $h^{-1}$) and logSMR (mg $O_2$ $h^{-1}$) = 0.6099 * (logmass) – 0.5988 (standard metabolic rate, log mg $O_2$ $h^{-1}$). All fish were included in these calculations.

Table 5.2A Model (GLM) summary for the effect of aerobic scope (AS, mg $O_2$ $h^{-1}$) on the competitive capacity in bullheads and stone loaches. Explanatory variables estimates (Parameters) and standard error (s.e.m). Reference species was bullhead and reference temperature was 13°C (n = 74).

| Term | d.f. | Mean squares | *F* | *p* | Parameters | s.e.m |
|---|---|---|---|---|---|---|
| Acclim temperature (13) | 2 | 70748 | 0.584 | 0.560 | | |
| (17) | | | | | 35.194 | 93.453 |
| (21) | | | | | -80.205 | 109.919 |
| logmass | 1 | 30723 | 0.254 | 0.616 | 76.336 | 445.437 |
| logAS | 1 | 10576 | 0.087 | 0.769 | 6.858 | 224.568 |
| Stone loach | 1 | 121051 | 0.999 | 0.321 | 104.425 | 104.455 |
| Residuals | 68 | 121121 | | | | |

*Interaction terms species*logAS was included in the original model but removed when not significant.*

Table 5.3B Model (GLM) summary for the effect of standard metabolic rate (SMR, mg $O_2$ $h^{-1}$) on the competitive capacity in bullheads and stone loaches. Explanatory variables estimates (Parameters) and standard error (s.e.m). Reference species was bullhead and reference temperature was 13°C (n = 74).

| Term | d.f. | Mean squares | *F* | *p* | Parameters | s.e.m |
|---|---|---|---|---|---|---|
| Acclim temperature (13) | 2 | 70748 | 0.586 | 0.559 | | |
| (17) | | | | | 49.78 | 98.55 |
| (21) | | | | | -53.20 | 124.14 |
| logmass | 1 | 30723 | 0.254 | 0.616 | 201.37 | 472.08 |
| logSMR | 1 | 12873 | 0.107 | 0.745 | -138.10 | 301.73 |
| Stone loach | 1 | 143936 | 1.192 | 0.279 | 110.93 | 101.60 |
| Residuals | 68 | 120751 | | | | |

*Interaction terms species*logSMR was included in the original model but removed when not significant.*

### 5.4.3 Temperature effect on metabolic traits between species

For metabolic rate trials, $MO_2$ values for 41 bullheads (at 13°C, n = 19; at 17°C, n = 14; at 21°C, n = 8, 10.13 ± 0.38 g) and for 45 stone loaches (at 13°C, n = 16; at 17°C, n =15; at 21°C, n = 14, 7.13 ± 0.26 g) were obtained. In bullheads, temperature showed no effect on MMR (GLM, $F_{2,40} = 1.715$, p = 0.194, all post hoc comparison cases p > 0.05). SMR did not increase with temperature (GLM, $F_{2,40} = 0.275$, p = 0.761, all post hoc comparison cases p > 0.05). Aerobic scope in bullheads was maintained over the range of temperatures tested (GLM, $F_{2,40} = 1.685$, p = 0.199, all post hoc comparison cases p > 0.05). A drop in AS was observed at 21°C by 22.29% compared to AS values at 17°C. For stone loaches, temperature did not affect MMR (GLM, $F_{2,44} = 1.986$, p = 0.15, all post hoc comparison cases p > 0.05). SMR increased significantly with temperature in stone loaches (GLM GLM, $F_{2,44} = 14.35$, p < 0.001, post hoc comparison, 13-17°C, p < 0.001, 13-21°C, p < 0.001, 17-21°C, p = 0.789). AS of stone loaches was maintained over all temperatures tested (GLM, $F_{2,44} = 2.926$, p = 0.065, all post hoc comparison cases p > 0.05). At 21°C, AS in stone loaches declined by 15.15% compared to AS values at 17°C. Stone loaches showed higher MMR values than bullheads (GLM, effect of species, $F_{1,85} = 13.628$, p < 0.001). No difference in SMR between two species was found (GLM, effect of species, $F_{1,85} = 2.873$, p = 0.094). Stone loaches displayed higher AS than bullheads (GLM, effect of species, $F_{1,85} = 8.450$, p = 0.005) (Figure 5.3).

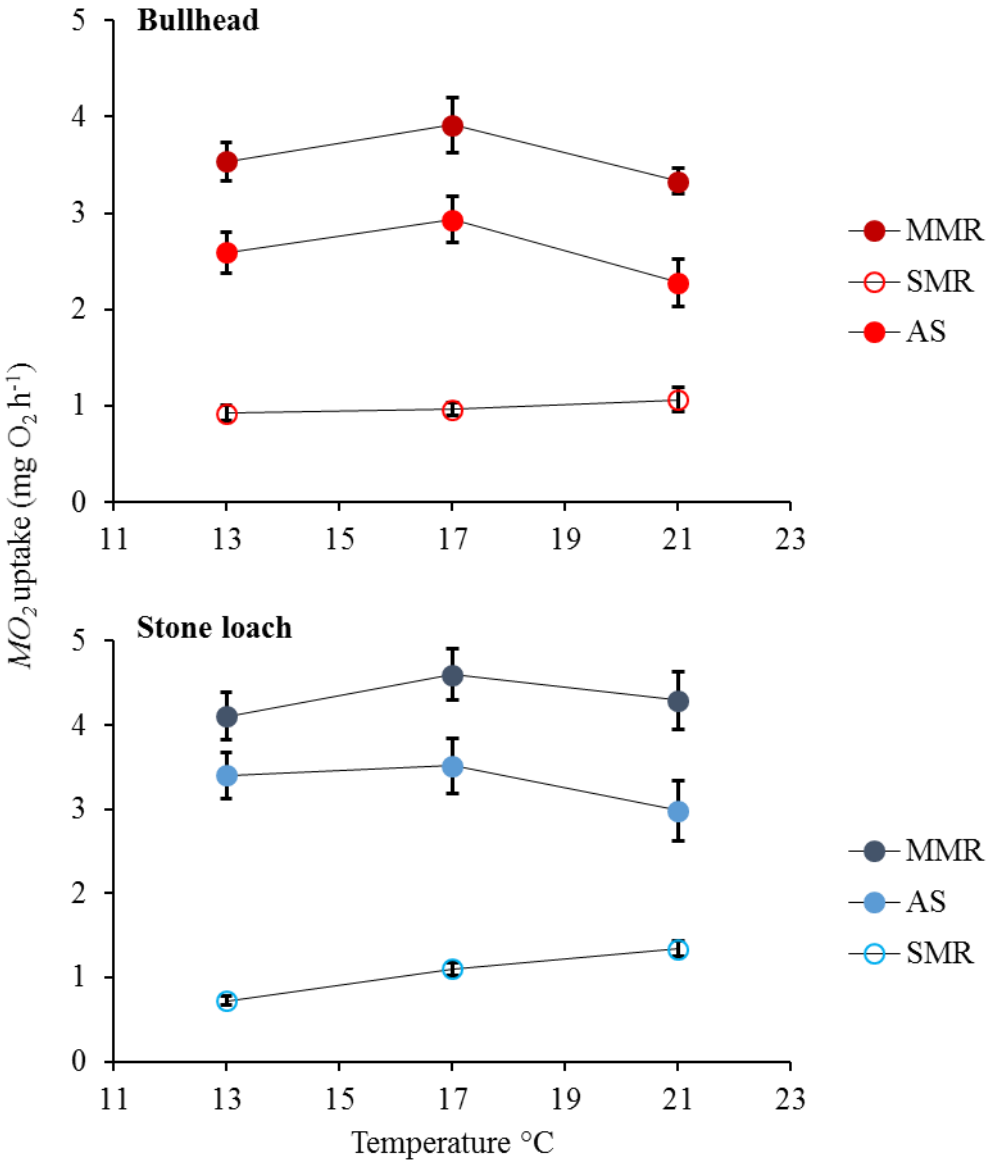



Figure 5.3 Oxygen uptake ($MO_2$, mg $O_2$ $h^{-1}$) over temperature (13°C, 17°C and 21°C), red dots representing SMR, MMR and AS. Bullhead $MO_2$ data (red dots) are on the top graph as for stone loach $MO_2$ values are represented on bottom graph (blue dots). All metabolic rates were corrected for the mean body mass (g) of all fish used during trials (8.56 ± 0.28 g) using residuals from regression of log SMR, log MMR and log AS versus log body mass derived equations for SMR (mg $O_2$ $h^{-1}$) = 0.711 * log(8.56) - 0.6872, for MMR (mg $O_2$ $h^{-1}$) = 0.7399 * log(8.56) - 0.1089 and for AS (mg $O_2$ $h^{-1}$) = 0.7871* log(8.56) - 0.2968. Data are represented in means ± s.e.m and are not log-transformed.

## 5.5 Discussion

### 5.5.1 Interspecific differences in competitive ability over different temperatures

Despite variation in competitive ability within each species, the results here suggest that stone loaches were likely able to outcompete bullheads for shelter across all temperatures tested and especially at the coldest temperature (13°C, Figure 5.1). Given the highly territorial behaviour of bullheads (Smyly 1957; Pecl 1990), it was surprising to observe that they had a lower competitive ability than stone loaches. Bullheads are known to actively defend acquired shelter against intruders by showing dominance behaviours and sometimes even engaging in aggressive fighting (Tomlinson & Perrow 2003). Additionally, bullheads prefer not to share the protective shelter with any conspecifics or another species (Symly 1955; Prenda et al., 1997a). In this study, no aggressive behaviour was observed between the species – individuals would generally exclude competitors from the shelter or other areas passively or by relatively benign pushes. At 17°C, both species displayed equal interest and competitive ability for a single shelter and occasionally even occupied the shelter simultaneously.

It has been suggested that bullheads and stone loaches display mutual avoidance behaviour and co-exist in rivers by the means of habitat partitioning (Welton et al., 1983; Welton et al., 1991), particularly at high population densities (Prenda et al., 1997b). In the wild over their native range, it has been observed that bullheads and stone loaches prefer to avoid each other (Smyly 1957; Prenda et al. 1997a); if bullheads are highly abundant, stone loaches are absent or rarely found and vice versa (Mann 1989; Yeomans, pers. comments). Prenda et al. (1997a) concluded for these two species, intraspecific competition for shelter is more pronounced than interspecific competition, especially for bullheads given their territorial behaviour (Davey et al., 2005). In the current study, competitive interactions occurred at low densities and at a relatively small spatial and temporal scale. It is therefore possible that the nature of the interactions observed here differed from those occurring in the wild. At 21°C, competitive ability or desire to use shelter decreased in both species. It is likely that at warmer temperatures both species cease to compete for shelter. This results were in contraction with previous findings (Carmona-Catot et al., 2013), who found a synergistic effect of temperature on the competitive outcomes for food availability between mosquitofish and toothcarp with mosquitofish outcompeting the native toothcarp at

warmer temperatures. In the current study no clear causal direction effect of temperature on competitive outcomes was observed.

The observed outcomes may be linked to other behavioural traits (e.g. as activity levels) or trade-offs between avoidance behaviours (e.g. competitor versus predator risk). At the coldest temperature, 13°C, both species were less active (see results in Chapter 4). Due to low activity levels at 13°C, stone loaches took the advantage of the shelter availability to minimise its predation risks. While bullheads were excluded from the shelter which may indicate that bullheads' risk of predation was higher at the colder temperatures compared to stone loaches. At the warmest temperature 21°C, both species cease to compete for shelter. At this temperature, activity levels increased in both species in particular in stone loaches (see results in Chapter 4). This may indicate that stone loaches might have chosen to actively swim rather than using the available shelter.

### 5.5.2 Links between metabolic traits and competitive capacity between and within species over different temperatures

Trade-offs between different behavioural responses to temperature change (competitive behaviour and shelter use versus activity) might be correlated to species or individual metabolic traits. An individual or a species with high aerobic capacity might have more flexibility in allocating sufficient energy to different behavioural traits simultaneously. In the current study, however, no link was found between SMR or AS and competitive ability among individuals within either species (Figure 5.2). This is in contrast to previous studies which have observed positive associations between intraspecific dominance or aggression and metabolic traits at the intraspecific (see details Biro & Stamps 2010; Killen et al., 2014; Metcalfe et al., 2016a) and interspecific levels (Seth et al., 2013). Positive intraspecifc correlations found between competitive behaviours (dominance or aggression) and metabolic traits were mainly observed in salmonids species that are known to undergo highly intense intraspecific hierarchy dominance interactions. Species or individuals with high baseline aggressive or dominant behaviours might require a higher aerobic capacity to facilitate physically intense behaviours. Yet here in this case, both species engaged in passive exclusions without aggressive chasing or attacks. As being both benthic species and having a sedentary lifestyle, it was less likely to observe a correlated relationship between metabolic traits and competitive behaviour for shelter on the intra and interspecific levels.

### 5.5.3 Temperature effect on metabolic traits between species

Bullheads and stone loaches displayed the similar trends for changes in AS with acclimation temperature (Figure 5.3) as seen in previous results in Chapter 4. However, there was evidence that the AS of bullheads was declining toward the upper range of the temperatures investigated in the current study. Stone loaches had a higher AS than bullheads over all temperature tested and showed less pronounced decline in AS at the warmest temperature tested. Having a higher AS might give stone loaches more flexibility in their energy allocation to physiological processes. A high aerobic metabolism can be energetic advantageous under favorable environmental conditions. While under unfavorable conditions (resources limitations) maintaining high aerobic demands can lead to energetic trade-offs (Reid et al., 2012).

### 5.5.4 Conclusions

This study examined competitive dyads in an attempt to understand how temperature may modulate links between physiology and behaviour within and between species. However, more complex interactions are likely to arise in the wild where competitive outcomes between native stone loaches and invasive bullheads for shelter are influenced by density-dependent processes. The outcomes of interspecific competition between native versus invasive fish species might as well depend on the aggressive nature of invader. If the invasive fish species is a highly aggressive competitor compared to native fish species (Dubs & Corkum 1996; Carmona-Cotat et al., 2013; Grabowska et al., 2016), the invasive fish species will outcompete or modify native fish species behaviour depending on the environmental and competitive context (e.g. competition for prey or cover). In the current study, bullheads and stone loaches might have excluded each other from the shelter relatively passively while direct attacks or bites were extremely rare. Interspecific competitive interactions might change and evolve according to environmental context and over different environmental stressors tested (e.g. temperature, $CO_2$ levels) (Killen et al., 2013). Consequently the competitive outcomes and interactions between two species, invader and native species may depend on; 1) initial competitive behaviour (territorial, dominance or aggression) of each species, 2) changes of behavioural responses towards different environmental stressors encountered in both species; 3) on the competition context (prey and/or cover availability) and finally on overall changes and differences in

metabolic capacity of each species independently if there are linked or not with behavioural changes.

In conclusion, invasive bullheads weren't capable of consistently outcompeting stone loaches for access to shelter and, if anything, actually showed lower competitive ability than stone loaches across a range of temperatures. Competitive ability was not linked to metabolic capacity (SMR or AS) among individuals as well between bullheads and stone loaches. No clear causal effect of temperature on the competitive interactions between stone loaches and bullheads were found. This would suggest that the interactions between these two fish is unlikely to change with the current trends in environmental warming. Furthermore, it is improbable that bullheads might displace stone loaches into less suitable habitats with rising temperatures. Other biological features such density population might have been more relevant in the interactions between bullheads and stone loaches.

# Chapter 6: Physiological and behavioural responses to hypoxia in an invasive and native freshwater fish species

Currently in preparation for submission to Journal of Experimental Biology

## 6.1 Abstract

Invasive species are considered to be one of the major environmental changes affecting biodiversity and provoking declines in native species. The mechanisms that allow certain species to become successful invaders are still poorly understood. Nevertheless, one characteristic defining a good invader is the capacity to cope and thrive in disturbed environments. In unfavourable conditions, such as during hypoxic episodes, invasive species might be better adapted and flexible in their physiological and behavioural responses towards this stressor. In this study, I compared physiological (e.g. metabolic rate, $P_{crit}$ value) and behavioural traits (avoidance behaviour with a shelter opportunity provided in hypoxic conditions) between two fish species: freshwater bullhead (*Cottus gobio*; invasive) and stone loach (*Barbatula barbatula*; native) over different dissolved oxygen (DO) concentrations in the water (100%, 80%, 60%, 40%, 30%, 25% and 20% DO levels) to look at the coping capacities between these species in hypoxic conditions. The $P_{crit}$ value for bullheads was established to be at 4.96 mg $O_2$ $l^{-1}$ at 14°C. Avoidance behaviour towards progressive hypoxia was similar between bullheads and stone loaches. Both species spent most of time hiding under the shelter in hypoxic conditions (bullheads: 100%; stone loaches: 93.93-99.73%). Stone loaches showed short and abrupt excursions into normoxic condition (6% of time spent at 25% DO level). I concluded that 1) stone loaches might be more resistant toward progressive hypoxia since no $P_{crit}$ was found. I hypothesised that this might be due to their secondary respiratory system (storage air in intestine system). 2) Bullheads stayed under severe hypoxic conditions hiding under the protective shelter independently of the metabolic constraints involved. This study suggests that the aerobic physiological attributes of invasive bullheads might be less well adapted to progressive hypoxia than those of native stone loaches. However, bullheads' behavioural response towards hypoxia was flexible and independent of aerobic metabolic limitations.

## 6.2 Introduction

The frequency and severity of hypoxic events in aquatic systems are increasing globally (Diaz 2001; Diaz & Rosenberg 2008), with increasing water temperatures suspected to enhance localised hypoxic events in lentic and lotic waters (Poff et al., 2002; Ficke et al., 2007). The frequency of hypoxic events is generally more pronounced in freshwater systems due to factors such as respiration of plant and algal communities, thermal variation, ice cover and eutrophication. Many temperate freshwater fishes may be affected by localised hypoxic events (Graham & Harrod 2009) with negative effects on growth, swimming performance, reproductive output and physiological stress (Herbert & Steffensen 2005; Domenici et al, 2007; Richards et al., 2009).

Fish can live under hypoxic conditions for a limited time by changing their behaviour or by adjusting metabolic pathways (Schurmann & Steffensen 1997; Herbert & Steffensen 2005; Landman et al., 2005; Evans 2007; Chabot & Claireaux 2008; Herbert et al., 2011; Cook & Herbert 2012a, b; Cook et al., 2013; Dupont-Prinet et al., 2013; Speers-Roesch et al., 2013; Elshout et al., 2013; Lefevre et al., 2014; He et al., 2015). Pelagic migratory fish species tend to increase activity in severe hypoxic events to escape and find more favourable habitats (Domenici et al., 2000; Herbert & Steffensen 2005; Brady et al., 2009), whereas sedentary fish often reduce activity even further as means to reduce energy expenditure and oxygen demand. These differences in hypoxia avoidance behaviours between pelagic and benthic fish species are mainly due to differences in locomotor capacities, energy allocation trade-offs, and the increased risk of predator exposure associated with increased activity. From a physiological standpoint, standard metabolic rate (SMR; the minimum energy required to sustain life) remains stable under moderate hypoxia ( $> 2$ mg $O_2$ $l^{-1}$, DO levels, dissolved oxygen levels in the water). With decreasing DO levels in the water, however, fish will reach a threshold referred to as the critical oxygen partial pressure ($P_{crit}$) at which oxygen availability is limiting and insufficient to maintain the SMR of the fish. The measurement of $P_{crit}$ is often used as a benchmark to determine hypoxia tolerance in species. Hypoxia-tolerant species such as marine tidepool sculpins (Cottidae), for example, are known to have lower $P_{crit}$ values compared to those of less tolerant species (Mandic et al., 2009a, b; Speers-Roesch et al., 2013).

Hypoxic events can provoke significant disturbances to aquatic community structure and can provide opportunities for invasive species to colonise ecosystems (Jewett et al., 2005). This may be particularly true if a potential invader has the appropriate physiological and

behavioural attributes to tolerate restricted access to environmental oxygen. One of the traits increasing invasion success is to have a wide tolerance range for different environmental factors (Kolar & Lodge 2002), and it has been suggested that invasive species might be more tolerant to hypoxic events compared to native species (Jewett et al., 2005; Lenz et al., 2011), through means of adaptation in metabolic pathways (low $P_{crit}$) and changes in behavioural responses (reduced activity levels). Invasive species with higher hypoxia tolerance may be able to expand and colonise novel habitats where the conditions have become unsuitable for native competitors or predators. In fish, comparative studies of hypoxia tolerance between native and non-native species are still scarce (Morosawa 2011; Elshout et al., 2013). Examining the effects of hypoxia on metabolism and behaviour between native and non-native fish species will provide insight into the mechanisms of invasion success of introduced species.

In this study, I investigated metabolic and behavioural responses to progressive hypoxia in bullheads (*Cottus gobio* Linnaeus 1758) and stone loaches (*Barbatula barbatula* Linnaeus 1758). Stone loaches are native to Scottish freshwater river ecosystems whereas bullheads have been introduced and considered to be invasive (Maitland & Campbell 1992). Both species are benthic and are known to occupy the same ecological niche and potentially compete for different resources (e.g. food and shelter). Like most temperate freshwater systems, Scottish rivers and streams experience seasonal low dissolved oxygen events correlated with flow, inorganic substances and temperature (Anderson et al., 2005). The invasion success of bullheads in their non-native range might be linked to higher tolerance range towards environmental stressors, such as hypoxic episodes, as compared to native stone loaches. Furthermore, bullheads belong to the order of Cottidae (sculpins) for which studies have shown in marine conspecifics to be moderately to highly tolerant towards hypoxia (Mandic et al., 2009a, b; Speers-Roesch et al., 2013), leading to the hypothesis that bullheads might have a higher hypoxia tolerance adaptation than native stone loaches. Specifically, the two main questions in this study were: 1) Are bullheads more hypoxia tolerant (i.e. have a lower $P_{crit}$) than stone loaches? and 2) Do bullheads and stone loaches differ in their hypoxia avoidance behaviour?

## 6.3 Methods and Materials

### 6.3.1 Animals and holding conditions

Fish were collected by electrofishing from the White Cart water catchment near Pollock, Scotland. Bullheads and stone loaches are both present and co-existing in this water catchment. Immediately after fish were caught, they were transported by road to Institute of Biodiversity, Animal Health and Comparative Medicine (IBAHCM) in Glasgow, Scotland, United Kingdom. After arrival, bullheads and stone loaches were separated and kept in different holding tanks of each 92 L (L = 620 mm, W = 620 mm and D = 240 mm). Tanks received a continuous freshwater flow through system connected to a main UV-steriliser and dechlorination unit. Water temperature in each tank was maintained at 14°C (± 0.5°C) and the photoperiod in the aquarium was set to a 12:12 light:dark cycle. Fish were fed daily *ad libitum* with frozen bloodworms and once a week with chunks of small fish to avoid a lack of protein in their diet. Three months before experiments, all fish (45 stone loaches and 42 bullheads) were lightly anaesthetised using benzocaine and marked for identification using coloured VIE elastomer tags (Northwest Marine Technology Inc.). The fish were then allocated to 5 stone loaches per tank (47 L, L = 520 mm, W = 380 mm and D = 240 mm) and 6 bullheads per tank (47 L, L = 520 mm, W = 380 mm and D = 240 mm or 30 L, L = 380 mm, W = 380 mm and D = 210 mm). Each of the holding tanks contained gravel substrate, plastic plants and circular plastic pipes to provide shelter. All experiments conducted during this study were in compliance with Home Office legalisation (Project Licence number: 60/4461) in United Kingdom

### 6.3.2 Respirometry

Oxygen uptake rate ($MO_2$, oxygen uptake measurements) of fish was measured using an intermittent-flow respirometry system following guidelines provided by Steffensen (1989) and Clark et al. (2013). One glass chamber (163 ml total volume) was submerged in a black experimental tank of 93 L (L = 780 mm, W = 570 mm, D = 210 mm) with air-saturated water. Temperature within the experimental tank was controlled by a thermostatic reservoir connected to the experimental tank by a thermoregulator (TMP-REG system, Loligo Systems, Tjele, Denmark) and maintained constant at 14°C (± 0.2°C) during the whole measurements period. To avoid an oxygen gradient in the respiromerty chamber, a continuous mixing circuit (100 ml/min) was induced by a peristaltic pump (Masterflex L/S 100 RPM, Cole-Parmer). An Eheim flush pump connected to an automated digital timer (MFRT-1, Superpro Hydroponics) flushed the respirometry

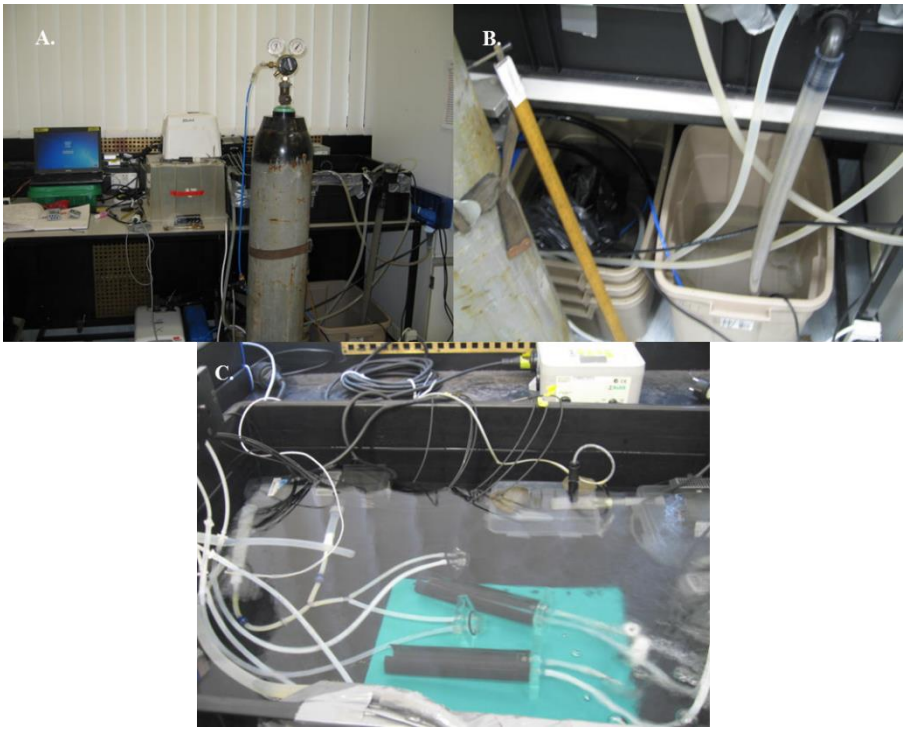
chamber for 3 min every 13 min with surrounding oxygenated water from the experimental tank. A UV filter-steriliser was connected to the experimental tank to minmise bacterial respiration during the trials. Oxygen concentrations in chamber was measured every two seconds using a Firesting 4 channel oxygen meter unit and associated sensors (PyroScience GmbH, Aachen, Germany). During the off cycle of 10 min, oxygen respiration rates ($MO_2$) were measured by the decline in oxygen concentration in the respirometry chamber. During the trials under normoxic conditions, the experimental tank was covered with black plastic shield to avoid any external disturbances.

To control the dissolved oxygen concentration (DO levels) in the water of the experimental tank, a galvanic oxygen probe (MINI-DO galvanic cell $O_2$ probe, Loligo Systems, Tjele, Denmark) was submerged and attached to Eheim pump maintaining a constant water flow over the probe membrane (see photo C.). The oxygen probe was connected to an oxymeter unit (OXY-REG, Loligo Systems, Tjele, Denmark) which can be set to maintain at the desired level of oxygen in water. The oxymeter controlled a solenoid valve (Loligo System, Tjele, Denmark) connected to a nitrogen tank. If levels of DO in the experimental tank went above the preset levels on the oxymeter unit (+ 1%), the solenoid valve would switch on and nitrogen was bubbled in reservoir tank and stopped when desirable preset DO levels were obtained. This reservoir tank was positioned under the experimental tank and constant water flow was maintained between experimental tank and reservoir tank, thus controlling the DO levels in experimental tank (see photos A. and B.). To maintain the DO levels at desirable oxygen concentrations in the experimental tank, manual adjustments were preformed between the amounts of oxygen pumped in the tank. During the hypoxic experiments, the black shield covering the experimental tank was removed in order to monitor fish behaviour in the chambers as oxygen levels declined (see below photos for experimental setup).

Ten stone loaches (7.16 ± 0.62 g, 9.39 ± 0.27 cm) and ten bullheads (10.99 ± 0.60 g, 8.33 ± 0.11 cm) were randomly selected from their holding tanks and tested individually under normoxic and hypoxic conditions to obtain oxygen uptake rates ($MO_2$). Before experiments, all fish were food-deprived for 48 h. Fish were firstly transferred individually in a circular tank (D = 50 cm) with a water depth of 10 cm and manually chased until exhaustion (Killen et al., 2012; Clark et al., 2013; Killen et al., 2015 see Appendix A2). Immediately after exhaustion (always after 2-3 min of chasing), fish were placed individually in a respirometry chamber. The manual chase protocol was chosen as both of these fish species are benthic and incapable of performing sustained swimming. After fish

have been transferred into the chamber, the first slope of $MO_2$ of oxygen decline (10 min) within the respirometry chamber was used to calculate the maximum rate of oxygen uptake MMR (MMR, in mg $O_2$ $h^{-1}$) followed by undisturbed 24 h period to obtain standard metabolic rate (SMR) under normoxic conditions. MMR was only measured under normoxic conditions. Standard metabolic rate (SMR, in mg $O_2$ $h^{-1}$) was calculated by taking the lowest 10% percentile of values during the whole measurement period (Dupont-Prinet et al., 2010; Killen et al., 2012; Killen 2014) excluding the first 5 h of measurements in the chamber when the oxygen uptake was elevated due to handling stress (Killen 2014). To account for bacterial respiration during the trials, background consumption was measured before and after each trial in the respirometry chamber.

The next day, measures of $MO_2$ during exposure to hypoxia were performed. Measurements started at 100% and DO levels were progressively lowered. Measurements were made at each level of oxygen availability for 1h 20 min, to obtain 6 measurements of oxygen uptake at each level of hypoxia. Following baseline normoxia measurements, oxygen concentration was lowered to the next desired DO level (80%, 60%, 40%, 30% and 25%) at a rate of 20% $h^{-1}$. At the end of the last slopes at 25% DO, the fish was removed from the chamber and transferred to its initial holding tanks. DO levels in the experimental tank were restored to 100% DO and blank bacterial background respirations were recorded. The following day, a new individual was tested following the same procedure.

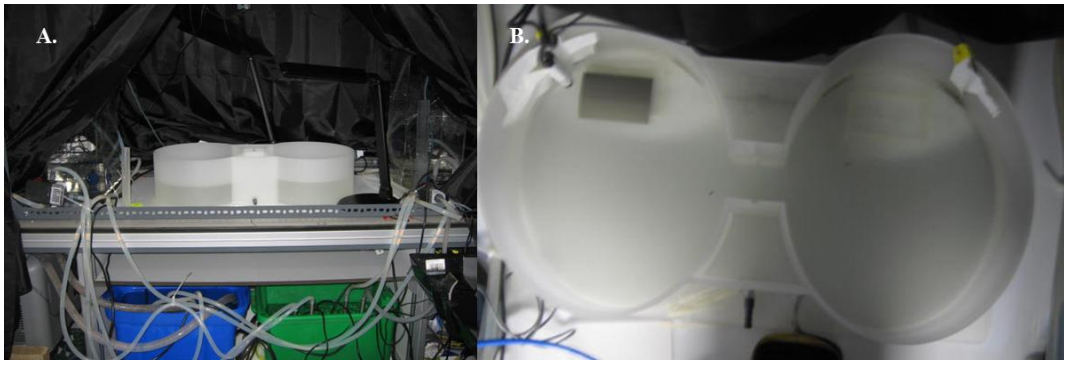



Photos of experimental setup for respirometry trials under hypoxic conditions.

### 6.3.3 Behavioural Assays

Behavioural responses of 11 bullheads (12.27 ± 0.90 g, 8.81 ± 0.20 cm) and 12 stone loaches (6.49 ± 0.40 g, 9.48 ± 0.20 cm) towards hypoxic conditions were performed in a shuttle-box system (Loligo Systems, Tjele, Denmark). The shuttle-box system consisted of an acrylic tank with two sides (diameter 30 cm on each side) connected by a small channel (L = 7 cm and W = 5.5 cm). Temperature on each side of the system was controlled and maintained at 14°C by a software (SHUTTLESOFT; Loligo Systems) during the whole trials (see Chapter 4 for more details). During the hypoxic conditions trials, only one side of the shuttle-box system was depleted to the desired amount of dissolved oxygen concentration in the water, whereas the other side was constantly 100% air saturated and serving as a potential refugia. Fish could freely choose between occupying normoxic or hypoxic conditions. Over the trials, allocation of hypoxic and normoxic sides were randomised. Water DO levels in the hypoxic side of the shuttle-box was controlled using a solenoid valve, bubbled nitrogen, and oximeter as previously described. Dissolved oxygen concentration levels (DO) in the water on both sides (hypoxic and normoxic side) were monitored and recorded continuously during the trials by two Firesting oxygen sensors each of both attached on the inflow water channel of each side. The sensors were linked to a Firesting 4 channel oxygen meter unit (PyroScience GmbH, Aachen, Germany) connected to a laptop computer. A shelter (L = 99 mm, W = 50 mm, H = 25 mm) was provided on the hypoxic side to encourage fish to stay in unfavourable conditions and observe a potential trade-off between hypoxia tolerance and willingness to take risk. The shelter was positioned between the inflow and outflow channels (~1.5 cm away from the behavioural walls). The induced circular water flow rate at each side of the shuttlebox system was not disturbed by the shelter (see photo B.). To record the behavioural response of fish to progressive hypoxia, a webcam was mounted above the behavioural arena. After 48 h without food, an individual fish was put randomly in one of the side of the shuttle-box approximately at 17:00 with a water depth of 7 cm and left undisturbed overnight in the behavioural arena. During this acclimation time, both sides of the system were set at 100% dissolved oxygen concentrations levels and maintained at 14°C. The next day at ~ 10:00, behavioural trials started at 100% of DO to obtain the baseline behaviour of fish under normoxic conditions. At each DO level (80%, 60%, 40%, 30%, 25% and 20%) behaviour was recorded for 30 min (and dissolved oxygen levels in the water were dropped at a rate of 20% per 15 min. At the end of each trial fish were taken out of the behavioural arena, measured for body mass and length, and returned to their initial holding tank. The

shuttle-box was cleaned and filled with clean water and a new individual fish was introduced in the arena to acclimate overnight (see photos below for experimental setup).




Photos of experimental setup for behavioural assays under hypoxic conditions.

## 6.4 Data and statistical analysis

### 6.4.1 Metabolic rate data

For metabolic rate trials, all $MO_2$ data were imported as text files obtained from the Firesting $O_2$ software and analysed in LabChart 7 Pro (ADInstuments Pty Ltd, Bella Vista, NSM, Australia). $MO_2$ (mg $O_2$ $h^{-1}$) data were corrected for the volume of respirometry chamber and volume of tubing in the closed-up system. $MO_2$ measurements were corrected for background bacterial respiration by assuming a linear increase in the background respiration over the time of measurements. From 10 min of initial slope of $MO_2$ measurements for MMR, I obtained 5 average slopes extracted over 2 min every two 2 min selections during the off cycle. From the 5 $MO_2$ slope data, I chose the slope with highest value to calculate MMR (in mg $O_2$ $h^{-1}$) for each fish. Aerobic scope (AS, in mg $O_2$ $h^{-1}$) was calculated as the absolute difference between MMR and SMR. Difference in metabolic traits (MMR, SMR and AS, in mg $O_2$ $h^{-1}$) at 100% air saturated water concentration between bullheads and stone loaches were tested by using GLM with mass of fish as continuous covariate and fish species as categorical covariate in the models. MMR, SMR and AS (in mg $O_2$ $h^{-1}$) and mass were log-transformed in the models.

At each DO level tested (80, 60, 40, 30 and 25%) the mean values of $MO_2$ data for 6 slopes recorded were calculated and used during the analyses for bullheads or stone loaches separately. To determine $P_{crit}$ in bullheads (n = 10) or stone loaches (n = 10) independently, a mixed effect linear model was used with $MO_2$ values (mg $O_2$ $h^{-1}$) at all DO levels as the response variable, DO levels as a categorical covariate (with 6 different levels), body mass (g) as continuous covariate and fish identity as random effect in the model. The $MO_2$

values (mg $O_2$ $h^{-1}$) and body mass were log-transformed in the models. The $MO_2$ data at normoxic level (SMR values at 100% DO levels) was considered as baseline reference in the model. Any DO levels for which $MO_2$ data were significantly below SMR values at 100% DO were used to determine the critical oxygen saturation point ($P_{crit}$) below which individual fish can't longer maintain the maintenance $O_2$ demands and regulate $O_2$ uptakes. Through these $MO_2$ data points a linear regression was plotted with a forced y intercept of zero, the resulting linear equation ($y = \beta x$, where $\beta$ is the slope of linear regression and y is the known SMR value in mg $O_2$ $h^{-1}$ at 100% DO levels, x represents the estimated $P_{crit}$ value) for the regression was used to calculate $P_{crit}$ for bullheads or stone loaches separately ($P_{crit} = y / \beta$, in % of dissolved oxygen levels DO in the water at 14°C) (Cook & Herbert 2012a).

### 6.4.2 Behavioural data

Videos of 11 bullheads (12.27 ± 0.90 g) and 12 stone loaches (6.49 ± 0.40 g) from behavioural trials were analysed with Solomon Coder software (v.14.10.04; Budapest, Hungary). To monitor and identify behavioural avoidance at each specific DO level measured (100, 80, 60, 40, 30, 25, 20% of dissolved oxygen in the water) in bullheads or stone loaches separately, residence times in hypoxic and normoxic sides (s, in sec), time spent under the shelter (s, in sec) in the hypoxic side were recorded. For each DO level, residence time in hypoxic and normoxic sides and time spent under shelter on the hypoxic side were calculated in percentage (%) and used for statistical analyses. Differences in behaviour (residence times in hypoxic and normoxic sides or time spent under the shelter tested all separately) compared to 100% DO level baseline reference behaviour within bullheads or stone loaches were analysed by using general linear models (GLMs) with DO levels as a categorical explanatory variable (with 7 levels). GLMs were followed with a Tukey HSD posthoc multiple comparison tests between the different DO levels, 100% DO levels was used as a reference baseline behaviour in each test. Differences in behaviour between bullheads and stone loaches were tested by performing a Welch two sample t test at each DO level.

Statistical analyses were performed in RStudio (version 3.3.0 The R Foundation for Statistical Computing Platform) with a significant level set at $p < 0.05$ using lmerTest package (Kuznetsova et al., 2015) and MuMIn package (Bartoń 2015) for calculating $R^2$ values of GLMM models; marginal $R^2$ indicates the variance explained by fixed factors, and conditional $R^2$ is the corresponding value for when including both fixed and random

factors (Nakagawa & Schielzeth 2013). Data represented in figures as mean values ± s.e.m otherwise stated.

## 6.5 Results

### 6.5.1 Metabolic traits

Stone loaches displayed a ca. 10.4% lower MMR (log MMR, 0.609 ± 0.04 mg $O_2$ $h^{-1}$) than bullheads (log MMR 0.68 ± 0.05 mg $O_2$ $h^{-1}$) at normoxia, but this difference was not statistically significant (GLM, effect species, $F_{1,19} = 2.16$, $p = 0.16$). Bullheads had a ca. 27% lower normoxic SMR (log SMR, -0.033 ± 0.019 mg $O_2$ $h^{-1}$) than stone loaches (logSMR, -0.125 ± 0.039 mg $O_2$ $h^{-1}$), but this difference was significant (GLM, effect species, $F_{1,19} = 12.75$, $p = 0.002$). There was difference in normoxic AS between bullheads and stone loaches (GLM, effect species, $F_{1,19} = 5.425$, $p = 0.032$), with bullheads showing a ca. 25% higher AS (log AS, 0.579 ± 0.025 mg $O_2$ $h^{-1}$) than stone loaches (log AS, 0.435 ± 0.053 mg $O_2$ $h^{-1}$).

For bullheads, $MO_2$ data values were significantly below SMR at 40% air saturation (0.801 ± 0.124 mg $O_2$ $h^{-1}$; $t = -2.061$, $p = 0.045$; Figure 6.1A; Table 6.1), 30% (0.590 ± 0.096 mg $O_2$ $h^{-1}$; $t = -5.634$, $p < 0.001$; Figure 6.1A; Table 6.1) and 25% DO levels (0.442 ± 0.095 mg $O_2$ $h^{-1}$; $t = -9.258$, $p < 0.001$; Figure 6.1A; Table 6.1). At 40% air saturation, SMR in bullheads dropped by 14.52%, at 30% DO SMR was 37.08% lower, when 25% DO levels were reached standard metabolic rate has declined by more than half (52.86%) comparing to initial values at 100% air saturated water. The GLMM model explained 78.69% of variation observed in changes in metabolic responses to hypoxia in bullheads; 65.38% of the total variation was explained by oxygen levels and body mass, and the remaining 13.31% of the explained variation was due to individual variation. $P_{crit}$ in bullheads was determined by forced intercept ($y = 0$) regression equation $y = 0.0195*x$, where x represents critical oxygen saturation point ($P_{crit}$), the intersection point between the regression line and extrapolated SMR value (here in this case y) at 100% air saturated water. The estimated $P_{crit}$ in bullheads was 4.96 mg $O_2$ $l^{-1}$ at 14°C (Figure 6.1A).

For stone loaches, only $MO_2$ values at 25% air saturation (0.593 ± 0.115 mg $O_2$ $h^{-1}$; $t = -4.273$, $p < 0.001$; Figure 6.1B; Table 6.2) were significantly lower than SMR values at 100% DO levels. At this point, fish became agitated and showed extreme bursts of activity, and the trials were halted. SMR values dropped by 10.55% at 30% DO levels and by

23.68% at 25% air saturated water concentration. As a result, no $P_{crit}$ value could be found for stone loaches (Figure 6.1B). The GLMM model relating variation in metabolic response in stone loaches towards hypoxia explained 80.44% of the observed variation. 55.91% was explained by oxygen availability and body mass, and the remaining 24.53% was attributed to individual differences in sensitivity to hypoxia.

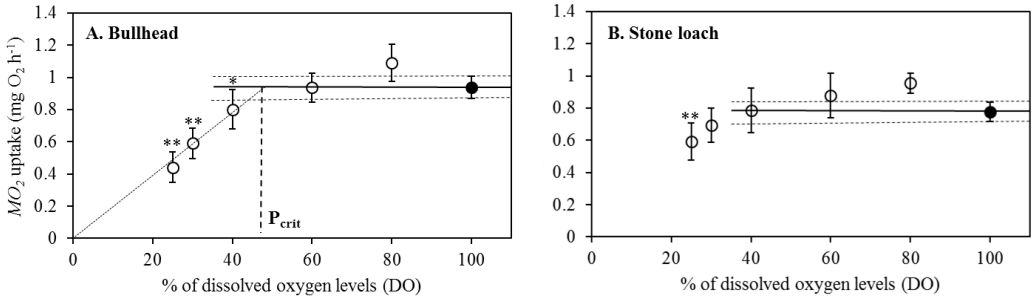



Figure 6.1 Respiratory responses in bullheads (A.) and stone loaches (B.) to progressive hypoxia at different dissolved oxygen levels (DO; 100, 80, 60, 40, 30 and 25%). The closed symbol represents SMR of bullheads (0.938 ± 0.068 mg $O_2$ $h^{-1}$) and stone loaches (0.777 ± 0.061 mg $O_2$ $h^{-1}$) under normoxia. The horizontal black lines are the mean value of SMR extrapolated over the range of DO levels tested with associated 95% C.I. values for SMR (dotted lines). Open symbols indicate values of $MO_2$ during progressive hypoxia levels. Linear regression (short dotted line) is plotted through $MO_2$ values significantly lower than defined SMR (with asterisks) with a forced intercept y = 0. Intercept point between regression line and extrapolated mean value of SMR represents $P_{crit}$ for bullheads (4.96 mg $O_2$ $l^{-1}$ at 14°C). For bullheads, metabolic rates were corrected for the mean body size (10.99 ± 0.60 g) using the residuals for regression of log SMR versus log body mass derived equation for SMR (mg $O_2$ $h^{-1}$) = 0.4942 * (10.99) – 0.642. For stone loaches, metabolic rates were corrected for the mean body size (7.16 ± 0.62 g) using the residuals for regression of log SMR versus log body mass derived equation for SMR (mg $O_2$ $h^{-1}$) = 0.7617* (7.16) – 0.7742. $MO_2$ uptake data represented in this figure is not log-transformed. Symbols and error bars are represented as mean ± 95% C.I.

### 6.5.2 Behaviour

All bullheads remained in hypoxia for 100% of time at each DO level tested; with 91.5-100% of time being spent under the provided shelter (Table 6.3) (all posthoc comparison cases; $p > 0.05$). Stone loaches similarly preferred to stay under hypoxic conditions over most trials (93.9 to 99.7% of the time); with 77.5-98.6% being spent in the provided shelter (all posthoc comparison cases; $p > 0.05$). In both bullheads and stone loaches, hypoxia had no effect on either the time spent in the hypoxia side of the arena or the time spent in shelter. No differences in residence time under hypoxia between bullheads and stone loaches were observed at most DO levels tested except at 20% air saturated water (Welch two sample t test; $t = 2.46$, $df = 11$, $p = 0.03$) where stone loaches spent less time in hypoxic conditions (96.25% of time) compared to bullheads. No differences were found for the time spent under the shelter in hypoxic side between bullheads and stone loaches at each DO level tested (all Welch two sample t test; $p > 0.05$).

Table 6.1 GLMM model summary for bullheads (n = 10) with response variables, standard metabolic rate log SMR (mg $O_2$ $h^{-1}$) and covariates as fixed effects, log mass (g) of fish and the different DO levels tested as intercept SMR (mg $O_2$ $h^{-1}$) values under normoxia. Estimate represent parameter estimates, s.e.m are standard errors, df are degrees of freedom, *t* and *p* values of statistical analyses. Random effect was represented by individual fish tested in the trials.

| **Fixed Effects** | **Bullhead** | | | | |
|---|---|---|---|---|---|
| | **estimate** | **s.e.m** | **df** | ***t*** | ***p*** |
| Intercept (log SMR mg $O_2$ $h^{-1}$ at 100%) | -0.545 | 0.322 | 8.09 | .1.691 | 0.129 |
| 80% | 0.062 | 0.038 | 45 | 1.661 | 0.104 |
| 60% | -0.008 | 0.038 | 45 | -0.202 | 0.841 |
| 40% | -0.077 | 0.038 | 45 | -2.061 | 0.045 |
| 30% | -0.212 | 0.038 | 45 | -5.634 | <0.001 |
| 25% | -0.348 | 0.038 | 45 | -9.258 | <0.001 |
| log mass (g) | 0.494 | 0.31 | 8 | 1.595 | 0.149 |
| **Random Effect** | | Variance | Std.Dev | | |
| Individual variation | | 0.004 | 0.066 | | |
| Residual | | 0.007 | 0.083 | | |

Table 6.2 GLMM model summary for stone loaches (n = 10) with response variables, standard metabolic rate log SMR (mg $O_2$ $h^{-1}$) and covariates as fixed effects, log mass (g) of fish and the different DO levels tested as intercept SMR (mg $O_2$ $h^{-1}$) values under normoxia. Estimate represent parameter estimates, s.e.m are standard errors, df are degrees of freedom, *t* and *p* values of statistical analyses. Random effect was represented by individual fish tested in the trials.

| **Fixed Effects** | **Stone loach** | | | | |
|---|---|---|---|---|---|
| | **estimate** | **s.e.m** | **df** | ***t*** | ***p*** |
| Intercept (log SMR mg $O_2$ $h^{-1}$ at 100%) | -0.764 | 0.183 | 8.21 | -4.182 | 0.003 |
| 80% | 0.083 | 0.032 | 45 | 2.600 | 0.013 |
| 60% | 0.051 | 0.032 | 45 | 1.604 | 0.116 |
| 40% | -0.0003 | 0.032 | 45 | -0.010 | 0.992 |
| 30% | -0.059 | 0.032 | 45 | -1.860 | 0.069 |
| 25% | -0.136 | 0.032 | 45 | -4.273 | <0.001 |
| log mass (g) | 0.762 | 0.214 | 8 | 3.556 | 0.007 |
| **Random Effect** | | Variance | Std.Dev | | |
| Individual variation | | 0.006 | 0.08 | | |
| Residual | | 0.005 | 0.071 | | |

Table 6.3 Residence time in percentage (% ± s.e.m) under hypoxic and normoxic conditions and time spent under shelter on hypoxic side of the shuttle-box system in bullheads (n = 11) and stone loaches (n = 12) at each DO level tested (100, 80, 60, 40, 30, 25 and 20% air saturated water).

| DO levels | Bullhead | | | Stone loach | | |
|---|---|---|---|---|---|---|
| | Hypoxia time | Shelter time | Normoxia time | Hypoxia time | Shelter time | Normoxia time |
| 100% | 100 | 91.54±8.46 | 0 | 99.73±0.23 | 98.61±1.25 | 0.27±0.23 |
| 80% | 100 | 100 | 0 | 97.83±1.83 | 95.24±2.47 | 2.16±1.83 |
| 60% | 100 | 100 | 0 | 99.08±0.60 | 96.21±2.42 | 0.92±0.60 |
| 40% | 100 | 99.90±0.10 | 0 | 97.47±1.40 | 93.47±3.68 | 2.53±1.40 |
| 30% | 100 | 98.75±1.25 | 0 | 96.19±2.44 | 87.35±6.55 | 3.98±2.43 |
| 25% | 100 | 98.38±1.27 | 0 | 93.93±3.34 | 81.91±9.06 | 6.07±3.34 |
| 20% | 100 | 92.88±6.73 | 0 | 96.25±1.52 | 77.46±9.42 | 3.75±1.52 |

## 6.6 Discussion

### 6.6.1 Are bullheads more hypoxia tolerant (lower $P_{crit}$) than stone loaches?

The results of this study suggest that, contrary to expectations, native stone loaches are more hypoxia tolerant than invasive bullheads. No $P_{crit}$ value could be found for stone loaches at 14°C. Stone loaches were able to maintain their SMR stable until 2.58 mg $O_2$ $l^{-1}$ DO level at 14°C. $P_{crit}$ in bullheads was estimated to be at 4.96 mg $O_2$ $l^{-1}$ DO level at 14°C. At 25% DO levels, SMR in bullheads dropped by approximately 50%, nearly double the decline observed for stone loaches at the same oxygen availability. Bullheads belong to the order of sculpins that are mainly found in marine habitats, are known to have moderate to high hypoxia tolerances (Mandic et al., 2009a, b; Speers-Roesch et al., 2013). However, Mandic and colleagues' studies (2009a, b) found that the freshwater sculpin species (*Cottus asper*) had one of the highest $P_{crit}$ values and therefore was less tolerant to hypoxic conditions compared to marine tide pool sculpins. These findings would suggest that freshwater sculpins might not have adjusted their physiological attributes towards hypoxia to the same extent as marine sculpin species. This would explain why bullheads had a higher $P_{crit}$ compared to marine sculpins in previous studies. Interestingly, some loach species have been observed to have some degree of tolerance to hypoxia. Eggs and larvae of spined loach (*Cobitis taenia*), can survive during acute exposure to oxygen concentrations of 2.1-2.2 mg $O_2$ $l^{-1}$ at 21°C (Bohlen 2003). Stone loaches in particular are able to respire through their intestines after ingesting air (Maitland 2007). In the current study, stone loaches were denied access to aerial oxygen during measurements of $P_{crit}$ (approx. 36 h), but it is possible that some individuals may have utilised a secondary

source of respiration. As I did not conduct bimodal respirometry trials (Lefevre et al., 2011, 2015), I was unable to determine the proportion of aerial respiration utilised during normoxic and hypoxic conditions in stone loaches. Therefore, it is practically impossible to establish how much stone loaches relied on their intestines as a secondary organ. Potentially invasive species benefit from having a wide physiological tolerance to environmental stressors. Contrary to initial expectations, the bullheads in the current study displayed a relatively low hypoxia tolerance. This would suggest that bullheads may not have been able to exploit hypoxic episodes as a mean to colonise or establish populations in novel habitats or expand their distribution range.

### 6.6.2 Do bullheads and stone loaches differ in hypoxia avoidance behaviour?

No difference in avoidance behaviour towards hypoxic conditions was found between bullheads and stone loaches except at the lowest level tested (20% DO) where stone loaches spent moderately less time under hypoxic conditions (96.25% of time) compared to bullheads which spent 100% of the time under hypoxia conditions over the whole trials. Stone loaches displayed short and abrupt excursions away from the shelter and into normoxic side of the behavioural arena with highest time spent in normoxia at 25% DO level. Despite these short explorative trips into normoxia, no statistically significant differences in behaviour compared to baseline behaviour under normoxia conditions could be found within stone loaches neither on time spent in hypoxia nor on time spent under shelter over all DO levels tested here in this study. Bullheads showed no avoidance behaviour toward progressive hypoxia and mostly stayed hiding under the provided shelter. Avoidance responses in fish to hypoxia vary according to the nature of hypoxic episode (exposure time, localised or generalised), lifestyle (migratory or sedentary), locomotion ability and opportunity of escaping routes to more favorable habitats. Benthic fish species as bullheads and stone loaches have very little swimming capacity and might use different behavioural strategies towards progressive hypoxia events. Exploring and finding more favorable environments can be energetically costly. Additionally, these novel habitats might be less suitable in terms of food and cover availability and have high predation risk than the exposed hypoxic habitats. For these reasons, some fish species might choose to stay in harsh hypoxic habitats, particularly if they are accustomed to relatively short periods of hypoxia (e.g. decreases in oxygen availability during daily cycling). Bullheads are known to be especially poor swimmers (Tudorache et al., 2008) and are very territorial

(Smyly 1957). Due to these characteristics, it might be energetically disadvantageous and highly costly for bullheads to invest energy into exploration and active strategies for avoiding hypoxia. Stone loaches showed similar behavioural responses towards progressive hypoxia as did bullheads, even though stone loaches tend to be more active and have higher energy requirements than bullheads (higher MMR, AS see Chapter 4 for details). This could explain the short explorative excursions into normoxia performed by stone loaches in order to quickly restore and maintain their high metabolic oxygen demands. Despite of having major metabolic constraints under hypoxia, bullheads stayed and secured their place under the protective shelter.

Differences in hypoxia tolerance between native and invasive species might be due to complex combinations between different strategies in energy allocations, physiological adaptation and behavioural compensation to maximise survival. Depending on factors such as competition for resources (interspecific and intraspecific) or the presence of predators, these compensatory strategies might vary and change. Stone loaches and bullheads differ in activity levels (stone loaches are more active, see results Chapter 4), peak levels of aerobic metabolism (higher MMR and AS for stone loaches, see results Chapter 4) and therefore may have different energy requirements to sustain these differences in behaviour and physiology when allowed to perform spontaneous activity. Bullheads, on the other hand, may rely more on anaerobic pathways (Mandic et al., 2013) than aerobic metabolism during hypoxic conditions. In this study, I did not investigate differences in anaerobic metabolic capacity between these two species, but this would be a promising area for future research. In the context of hypoxia tolerance between invasive and native fish species, looking at whole animal performance and behavioural traits such as metabolic rate and avoidance behaviour as shown in this study might not give a clear and complete representation of all the underlying physiological pathways involved in hypoxia resistance.

I suggest additional research is needed looking at lower level traits that may also explain differences in the physiological tolerance to hypoxia in these species such as hematocrit, oxygen binding capacity of hemoglobin and different levels or isoforms anaerobic enzymes in different relevant tissues (e.g. brain, liver) to understand why certain invaders are able to thrive in hypoxia. Overall more investigation is required into different native versus invasive species pairs, looking into tolerance ranges toward different environmental stressors, integrating physiology and behaviour over different environmental contexts.

# Chapter 7: Discussion

## 7.1 Main Aims and Objectives

The main aim of my PhD thesis was to examine how whole-animal aerobic capacity and behavioural traits may influence species distribution patterns and invasion success in fish. I performed a combination of broad comparative analyses and carefully designed laboratory experiments on native stone loach (*Barbatula barbatula)* and invasive bullhead (*Cottus gobio*) to address specific questions related to the major themes of my thesis.

In Chapter 2, by collecting aerobic scope data from the literature and performing a phylogenetically-informed analysis, I found no evidence for the direct implication of aerobic scope (AS) in influencing the currently observed distributions for 86 fish species. AS was also not related to invasion success in 59 freshwater fish species (23 invasive and 36 native species).

In Chapter 3, again using literature data for aerobic scope and conducting a phylogenetically-informed comparative analysis, I found no indication of trade-off between peak and thermal performance breadth for AS across 28 fish species.

In Chapter 4, I tested the physiological and behavioural responses of bullheads and stone loaches to acute and chronic temperature shifts. I found that both species were able to maintain AS over the same thermal range (13-21°C). Contrary to my initial hypotheses, bullheads had a lower maximum metabolic rate (MMR) and AS than stone loaches over all temperatures tested. Bullheads were also less active overall and preferred colder temperatures (17.5-19°C) than stone loaches (21-22.4°C).

In Chapter 5, I examined the competitive behaviour in bullheads and stone loaches, at three different temperatures (13°C, 17°C and 21°C). I demonstrated that overall, native stone loach was a better competitor for shelter use in particular at 13°C. There was no clear causal effect of temperature or AS on competitive outcomes between these two species. Furthermore, no links between competitive behaviour and metabolic traits were found among individuals within each species.

In Chapter 6, I tested the hypoxia tolerance and avoidance behaviour in bullheads and stone loaches over different dissolved oxygen (DO) concentrations (100%, 80%, 60%, 40%, 30%, 25% and 20% DO levels). I found that bullheads were less tolerant to hypoxic conditions with a $P_{crit}$ value of 4.96 mg $O_2$ $l^{-1}$ at 14°C. Avoidance behaviour towards progressive hypoxia was similar between bullheads and stone loaches; both species spent most of their time utilising the shelter even in hypoxic conditions. However, stone loaches showed short and abrupt excursions into normoxic water when exposed to severe hypoxia.

## 7.2 Chapter 2: The effect of metabolic traits on the geographic distribution and invasion success of teleost fishes

Aerobic scope has been proposed to be one of physiological constraints on geographical ranges in ectotherms (Pörtner, 2001; Pörtner & Farrell 2008; Pörtner & Gutt 2016). Previous studies in fish have found positive links between aerobic scope and latitudinal distribution (Gardiner et al., 2010, Naya et al., 2012b) showing that individuals or species at higher latitudinal positions or over larger distribution range possess higher aerobic capacities. Gardiner et al. (2010) demonstrated within several species of coral reef damselfish and cardinalfish from two source populations (Lizard and Heron Islands, 1200 km apart) that individuals found at high latitude position (Heron Islands) had higher aerobic scope performance compared to individuals at low latitude position (Lizard Island). Gardiner et al. (2010) results were mainly due to local adaptation possible by an increase of maximum metabolic rate (MMR) for the individuals from high latitude populations. This indicates a possible latitudinal variation in aerobic scope performance within species due to local adaptation towards thermal conditions. Though, the direct links of aerobic scope with distribution patterns across a set of different fish species has been largely overlooked.

Payne et al. (2016) proposed that aerobic scope might set the upper thermal limit of the habitable regions in nine different temperate and tropical species. Showing that a positive link between optimum temperature for aerobic scope and the highest temperature encountered in the range of teleost fish species exists. In addition, another study performed over a larger geographical range across 38 different fish species showed a positive correlation between aerobic scope and latitude distribution range (Naya & Bozinovic 2012). Although, Naya & Bozinovic 2012 reported the difference between RMR and SMR (termed as “metabolic scope”) and included a life history trait such trophic level in their models that might have biased or obscure the real effect of metabolic scope over latitude.

In Naya & Bozinovic (2012) study, no phylogenetic correction on the dataset had been made either. Till up to date, an ongoing debate on the relevance of aerobic scope in current distribution patterns in ectotherms is still continuing (Clark, Sandblom & Jutfelt 2013; Farrell 2013; Pörtner & Giomi 2013; Pörtner 2014; Wang et al. 2014; Farrell, 2016).

The findings obtained over my widely diverse phylogenetically-informed analysis will highly contribute to this ongoing debate and give more insights of the direct implications of AS in observed latitudinal trends in fishes. I did not find evidence of a direct effect of AS on latitudinal distribution across 86 fish species. This is the first study to perform a phylogenetically-informed analysis on the effect of AS on currently observed geographical distribution range across a diverse range of fishes. My results were in opposition with previous studies' findings (Naya & Bozinovic 2012; Payne et al., 2016) which found an association between what they termed "metabolic scope" and latitudinal patterns in fish. Notably, however, Naya & Bozinovic 2012 reported the difference between routine metabolic rate (RMR) and SMR and also provided no phylogenetic correction on their dataset. Using a combination of laboratory and field data, Payne et al. (2016) showed a positive link between optimum temperature for AS and the highest temperature encountered in the range of nine teleost fish species exists. These authors proposed that AS might set an upper thermal limit of the habitable ranges of fishes. There is an ongoing debate on the relevance of aerobic scope in current distribution patterns in ectotherms (Clark et al., 2013; Farrell 2013; Pörtner & Giomi 2013; Pörtner 2014; Wang et al. 2014; Farrell, 2016). My analyses contribute to this discussion by demonstrating that having a high AS does not necessarily result in a larger distribution range. It can thus be concluded that the current geographical ranges of teleost fish species are not constrained by AS, as has previously been suggested (Pörtner 2001; Pörtner & Farrell 2008; Pörtner & Gutt 2016).

Therefore, it may be plausible that fishes have the necessary capacity for plasticity to adjust their AS according to energetic needs and across environmental variation to enable them to extend their distribution range. One possible mechanism fish species may have, is the capacity to adjust their capacity for aerobic metabolism with warming, especially if compensatory processes (e.g. changes in membrane structure and function, or changes in concentration in isoforms or different types of aerobic enzymes) allow for rapid thermal acclimatisation. Nonetheless, it could be that the observed distribution trends in fish might be the results of other underlying physiological traits (e.g. mitochondria efficiency and cardiac functioning).

I found no evidence of a direct association between AS and invasion success in 59 freshwater fish species (23 invasive and 36 native species). In addition, invasive fish species in this dataset did not have higher AS values than native species as previously found in Marras et al. (2015). To my knowledge this was the first attempt to examine the direct effects of AS on invasion success over a broad range of teleost taxa. There is still need to collect AS data on additional invasive fish species to confirm this line of analysis. This is especially true for marine habitats – there is currently very few invasive marine fish species for which data is available for metabolic traits.

## 7.3 Chapter 3: Is there a trade-off between peak performance and performance breadth across temperatures for aerobic scope in teleost fishes?

My findings in Chapter 2 showed that peak AS was not linked with the geographical ranges of various teleost fish species. However, a possibility that could not be discounted was that there might be a trade-off between peak AS and the breadth of performance across temperatures, such that species with a lower AS might actually be able to function normally across a broad range of temperatures (i.e. there is a specialist-generalist trade-off for AS in fishes). In Chapter 3, I performed an in-depth investigation of this issue by focusing on those fish species for which there is AS data available in at least three temperatures.

This analysis revealed no evidence of a trade-off between thermal peak and breadth performance for AS across 28 species, suggesting that there is indeed no strict trade-off between being a thermal generalist versus a thermal specialist for AS in fishes. Consequently, prior adaptation to a wider thermal breadth of performance may not lead to a reduction in peak performance, at least for AS.

As seen from the results in Chapter 2, AS may not constrain the geographical distributions of fish as previously proposed (Pörtner & Farrell 2008). The findings in Chapter 2 coincide with the results obtained in Chapter 3. For example, tropical stenothermal species are known for having narrower thermal tolerance ranges than more generalist species (Sunday et al., 2011). Additionally, these tropical species may be able to adapt their thermal optima for AS (Munday et al., 2016) or expand their thermal breadth with current rates of

warming and possibly allow them to extend their distribution ranges. Yet, this does not seem to be the case in all tropical species. For example, *Chromis viridis* exhibit no plastic response in AS with warmer temperatures even after acclimation (Habary et al., 2016).

I also observed that peak performance for AS was higher in species with a higher optimal temperature for AS which correlated with the "hotter is better" model of thermal adaptation (Angilletta et al., 2010). These results complement the findings of Payne et al. (2016), who found that peak performance in nine temperate and tropical fish species correlated with their maximum temperatures encountered in their range. Payne et al. (2016) concluded that AS may determine the upper thermal limit of the habitable ecological niche in fish.

## 7.4 Chapter 4: Metabolic and behavioural responses to thermal variation in an invasive and native freshwater fish species

Stone loaches and bullheads showed little differences in their metabolic responses (MMR, SMR and AS) to either acute or long term variation in temperatures (13-21°C). Both species showed similar increases in SMR with temperature during both the acute and acclimated trials. In previous studies, lower SMR values in invasive species compared to native species have been observed, possibly contributing to the invasion success (Maazouzi et al., 2011; Becker et al., 2015). During unfavourable conditions a lower SMR could translate into a greater ability to survive periods of limited and unpredictable food or oxygen availability due to reduced maintenance requirements (Reid et al., 2012). This is unlikely to be a mechanism allowing bullheads to outcompete stone loaches given that the two species show vary similar values for SMR over a range of temperatures.

Stone loaches had a higher MMR and AS than bullheads during acute or acclimated trials. The differences in MMR and AS between species might be related to differences in foraging behaviour and swimming abilities. I also observed differences in night and morning spontaneous activity between bullheads and stone loaches, with stone loaches being generally more active than bullheads. Both species were more active during the night as compared to the morning, agreeing with previous observations of these species (Smyly 1955, 1957; Fischer 2004; Nunn et al., 2010). There was also a general positive effect of temperature on both overnight and morning activity (see Figure 4.2).

Both stone loaches and bullheads showed very little change in AS over the range of temperatures examined in the studies contained within this thesis. As such, there were no obvious differences in thermal optima for metabolic traits as found in previous studies examining differences in traits between native and invasive fish species (Maazouzi et al., 2011; Marras et al., 2015). The results in my studies may instead be more reflective of the scenario illustrated in Figure 7.1C, but with the invasive species, in this case the bullheads, exhibiting a lower AS over a broad range of temperatures. Overall, thermal effects on AS probably does not act as a contributing factor to invasion success of bullheads in Scotland.

Stone loaches preferred warmer temperatures (21-22°C) than bullheads (17.5-19°C) independently of acclimation temperatures. Across acclimation temperatures, SMR in both species was positively linked to temperature preference. Killen (2014) found the opposite trend in minnows *Phoxinus phoxinus* where individuals with a higher SMR tended to choose colder temperatures. He concluded individuals chose colder temperature to reduce maintenance costs and enable them to reallocate surplus energy to growth. For bullheads and stone loaches, which are comparatively more inactive and benthic-oriented species, choosing warmer temperatures may facilitate increased foraging activity or growth, provided that there is adequate food in the environment to support the increase in maintenance costs (Angilletta et al., 2010).

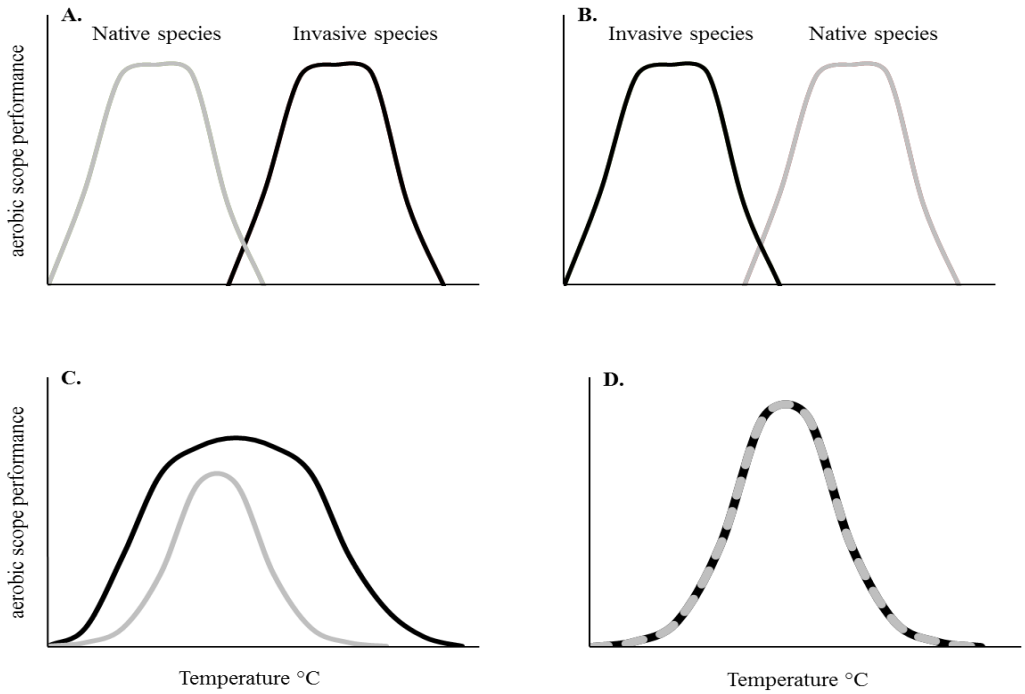



Figure 7.1 Different case scenarios of thermal performance curve (TPC) for aerobic scope between invasive (black curve) and native species (grey curve). A. Invasive species perform better at warmer temperatures than native species and maximised its aerobic scope at warm temperatures (Marras et al., 2015). B. It is the opposite case, where native species perform better at warmer temperatures than invasive species (Maazouzi et al., 2011) C. Invasive species has a higher and aerobic scope performance curve than native over a range of temperatures. D. No differences in thermal performance curves for aerobic scope is found between invasive and native species.

## 7.5 Chapter 5: The effect of thermal tolerance on competitive outcomes between an invasive and a native freshwater fish species

The results of this chapter suggest that bullheads are unlikely to outcompete stone loaches for available shelter and that, if anything, stone loaches may be the better competitor and especially at colder temperatures. Overall, temperature had no clear modulating effect on the competitive outcomes between these two species. The outcomes of competitive interactions between bullheads and stone loaches may instead be related to differences in perceived risk between the two species and trade-offs between hiding in shelter and actively foraging. Although I initially hypothesised that such behavioural responses (competitive ability versus activity levels) may be related metabolic traits at the individual or species levels, I found no such links between either SMR or AS and competitive ability.

## 7.6 Chapter 6: Physiological and behavioural responses to hypoxia in an invasive and native freshwater fish species

I found that invasive bullheads were less tolerant to progressive hypoxia conditions than native stone loaches. $P_{crit}$ value of bullheads was estimated at 4.96 mg $O_2$ $l^{-1}$ at 14°C. As for stone loaches, no $P_{crit}$ could be identified over the tested DO levels (80, 60, 40, 30 and 25% dissolved oxygen concentration) at 14°C. These findings were contrary to results from other studies that found a higher hypoxia tolerance in invasive species (Morosawa 2011). Interestingly, stone loaches possess a secondary breathing organ in their gut which they can use to store air (Maitland 2007). Although fish in this study were denied access to atmospheric oxygen during measurements of $P_{crit}$, the possibility can not be excluded that some fish were able to ingest air before placement into the chamber. As for another stone loach species (*Cobitis taenia*), it has been demonstrated that they can tolerate exposure to acute hypoxia at 2.1-2.2 mg $O_2$/l at 21°C (blastula stage to exogenous feeding larvae, Bohlen 2003).

Bullheads belong to the sculpins order (Cottidae) which are known to be tolerant to moderate to high levels of hypoxia (Mandic et al., 2009b; Mandic et al., 2013; Speers-Roesch et al., 2013). It was therefore surprising to observe that the freshwater bullheads appeared to be less tolerant to hypoxia as compared to their marine conspecifics. Notably, however, previous work has shown that one other freshwater sculpin (*Cottus asper*) has a similarly elevated $P_{crit}$ (Mandic et al., 2009a, b). This would indicate the freshwater sculpins might not have adjusted to the same extent their physiological attributes (e.g. affinity and carrying capacity of haemoglobin for oxygen). Hypoxia tolerance in sculpins has also been identified to be linked to high anaerobic enzymes activities (Mandic et al., 2013). Here in this study, I did not focus on anaerobic metabolic pathways. Comparisons of anaerobic capacity between bullheads and stone loaches would be a worthwhile area for future research, especially given that anaerobic metabolism is utilised during exposure to environmental stressors (e.g. hypoxia) as well as during predator-prey interactions (e.g. fast-start escape responses).

The difference observed in hypoxia tolerance between stone loaches and bullheads might differ in their gill morphology and respiratory surface area. Fish can vary and change their respiratory surface area according to their oxygen needs and according to environmental conditions (Nilsson 2007). As seen in results of chapter 4, stone loaches have a higher MMR and AS, therefore requires more oxygen uptake than bullheads with a lower AS.

Stone loaches may have a large respiratory surface area to sustain the tissues and organs required to support their higher MMR and AS. By possessing a larger respiratory surface area would allow for increase of water flux diffusing over the gills, therefore an increase in intake of oxygen by the gills. Nevertheless, a larger respiratory surface can also come with a osmoregulatory cost (e.g. loss of ions across the gills during times of high perfusion) (Nilsson 2007).

## 7.7 Project limitations

### 7.7.1 Methodological limitations

In Chapter 2, as the data used in this analysis was collected from literature for AS data and from FishBase for latitudinal range data, I faced several limitations. For absolute latitude range data used in this analysis, did not give me any clear indication on daily or seasonal thermal variations experienced by each of the species in the dataset. Furthermore, in the dataset of species I had, different fish species with different migration and dispersal patterns (vertical or horizontal migrations) may naturally experience high thermal changes during their daily or seasonal migrations routes compared to less mobile localised species. Oppositely, other species with no migrations or dispersal abilities and site fidelity behaviours may not even use the whole absolute range reported in my dataset. I could not account for these differences in migration trends and thermal variation experienced. One possibility to get an indication of thermal variability experienced by each one of species in the dataset, would be to extract temperature range of the site of collection in each of the studies. Nevertheless, this information is not always reported in the literature and this would still not exclude the difference in temperature range experienced by migratory fish. Recent studies have used telemetry methods to collect data on migratory fish relating migration patterns to physiology (Cooke et al., 2008). New telemetry devices (accelerometry) have been created to record oxygen consumption of an individual fish while facing different ecological and environmental challenges. This new technology could give us an insight of energy expenditures and allocation decision trade-offs in fish over different biological contexts (migrations, competitions, predations) (see for details Metcalfe et al., 2016b).

As for the AS data used in this analysis in Chapter 2, may not necessarily reflect the respective AS peak performance for each species. For the majority of fish species, AS data were extracted from studies which measured AS at one test temperature (n = 52). If

optimum temperature for AS may exist in these cases, there is probably a chance peak performance for AS may not have been recorded. Furthermore, as AS can change with temperature in different ways in many species (see for details Lefevre 2016), this may have cause a bias in the attempt to find an accurate relationship between latitudinal range and AS across species due to differences in adapting to thermal variation. Even though, I could not find a relationship between thermal peak and breadth for AS across 28 fish species, there are definitely variations and differences in thermal performance curve for AS tested against temperatures.

This leads me to the limitations experienced for the data analysis in Chapter 3. As mentioned above most studies up to date have measured AS over limited number of temperature points to allow for complex asymmetrical modelling (Angilletta 2006). Certain fish species as well do not display bell shaped thermal performance curve for AS and therefore could not be used in the analysis (15 species). For these particular species, no significant drop of at least 90 % in AS performance neither above nor below optimum temperature ($T_{opt}$) for AS was observed, therefore these species were excluded from the analysis. Included in these set of excluded species were the AS performance data collected for bullheads and stone loaches in Chapter 4 and 5 as no significant drop was observed in both species. Nevertheless, the acclimated AS performance data for bullheads and stone loaches collected in Chapter 5 at 17°C were used in the absolute latitudinal and invasiveness analysis performed in Chapter 2. I chose the AS performance data at 17°C as it seems to be the temperature at which AS peaked in bullheads and stone loaches (see results in Chapter 5, see Figure 5.3).

### 7.7.2 Study species

Over chapters 4-6, I investigated differences in physiological (AS) and behavioural traits over different environmental conditions (e.g. temperature and hypoxia) in bullheads and stone loaches to understand bullheads' invasion success in Scottish rivers. I tested the competitive behaviour for shelter between both species and how these interspecific interactions may change over environmental stressor such as temperature. The results I found over the different chapters are: 1) bullheads have lower AS as stone loaches over the same thermal range (13-21°C), 2) they are less active and preferred colder temperatures (17.5-19°C), 3) they have low competitive ability for shelter than stone loaches and 4) bullheads' $P_{crit}$ was at 4.96 mg $O_2$ $l^{-1}$ DO levels at 14°C. It is unlikely that bullheads will displace stone loaches into less suitable habitat with further warming due to their

differences in aerobic capacity, activity levels, competitive ability and temperature preferences. It might be possible that bullheads rely more on anaerobic pathways, in particular during periods of harsh conditions (e.g. hypoxia). In marine sculpins species, it has been suggested that an increase in anaerobic enzyme activities in brain is associated with hypoxia tolerance in these species (Mandic et al., 2013). I did not look into the anaerobic metabolism in both species during my thesis. It could be plausible both species have different proportion usage between aerobic and anaerobic metabolism due to differences in activity, foraging behaviour and hypoxic tolerance mechanisms.

All experiments conducted over the course of this PhD project were conducted in controlled laboratories settings. The results obtained during these sets of experiments might not necessarily reflect the outcomes observed in the wild. In the Clyde catchment tributaries, a continuing increase in bullheads population numbers has been observed over the last 15 years (Yeomans pers.comments). Furthermore, bullheads are actively spreading and extending their invaded distribution range (Yeomans & Jennings 2013). During the annual fishing and fish populations companies done by the Clyde River Foundation, they observed a general trend in the rivers where both bullheads and stone loaches should be co-existing, the numbers of stone loaches are dropping or not present. It has been speculated that the increase in bullheads populations might have caused this phenomenon. However, no clear conclusions could have been drawn about the particular ongoing interactions between these two fish species. The next step in this research focus would be to collect field metabolic rates on both species as well the collection of life history and behavioural traits (dispersal capacity, competitive ability) under the actual species' density population conditions. In the case of bullheads and stone loaches, the intra and interspecific interaction might possibly be density dependent.

In research, it is common that caught wild fish species might not handle well the captive conditions depending and varying according to the fish species. Certain individual fish can develop some diseases and infections which might have already been present under wild conditions. However probably due to stress induced by transportation and new conditions, this might have caused a disease outbreak and eventually might lead to high mortality in captivity. During my experiments in Chapter 4, I encountered some disease cases in my bullheads and a few mortalities at the warmest temperatures treatments (17 and 21°C) for bullheads. Stone loaches were not affected and seemed to be more robust to temperatures treatments. One bullhead died over the cross of the acute thermal exposed metabolic rates trials. In this case, this individual bullhead was not included the analysis. I can not exclude

the eventuality that some of my bullheads might have been affected by internal bacteria or internal disease without showing external symptoms, therefore I could not directly reject the possibility that this might have reflected on the results obtained during the analyses in Chapters 4 and 5. Although, I am clearly positive that the physiological responses I obtained during the experiments in Chapters 4 and 5, are not stress physiological responses in both fish species. The range of temperature tested (13-21°C) during this project was out of the range of temperatures which induce physiological stress responses. In adult bullheads, the temperature at which feeding cease, was set at 26.5°C and in adult stone loaches it was at 28°C (Elliott et al., 1994, 1995). This concludes that the recorded metabolic rates under controlled laboratory settings in this project, reflected the oxygen consumptions over the natural thermal range experienced in the wild for both fish species during the summer season.

During this PhD project, I was limited by one pair of species, invasive bullheads versus stone loaches to test my hypotheses on. Due to logistic issues, I was unable to obtain other pair of fish species. As recent literatures emphasised on the importance to include physiological tools and methodologies in the invasion ecology domain (Cooke et al., 2013; Lennox et al., 2015), this project sets a basic framework on the importance and role of physiological traits in the invasion process in one specific invasive species. One of the general aim of this project was to contribute to the general understanding on 1) how whole-animal trait performance such as AS is involved in the invasion process and success of an invasive species and 2) how differences in metabolic and behavioural traits between native and invasive species might have favoured the invader to the extent of the native species. Recent studies have looked in differences in thermal performance curve of AS between invasive versus native species. Marras et al. (2015) found differences in thermal range for AS in invasive and native fish species. Invasive marbled spinefoot (*Siganus rivulatus*) had a higher AS than native salema (*Sarpa salpa*) and aerobic scope performance peaked at warmer temperature (29.1°C) compared to the native salema (21.8°C). For competitive behaviour, Carmona-Catot et al. (2013) found that the invasive mosquitofish (*Gambusia holbrooki*) outcompeted native toothcarp (*Aphanius iberus*) for food resources at warmer temperatures. To summarise, differences in metabolic and behavioural traits between native and invasive species and competitive interspecific outcomes may depend on: 1) initial competitive behaviour (territorial, dominance or aggression) of each species, 2) reaction norms each of these traits in regard of environmental stressors and on 3) competition context. More research is needed to look into these different aspects in other competitive pairs of native versus invasive species.

## 7.8 Future Directions

### 7.8.1 The role of physiology in distribution patterns

The literature surveys conducted for Chapters 2 and 3 illustrate a pressing need for more studies that measure AS in fish over at least 5-6 temperature points in order to: 1) perform complex curve modelling methods to obtain more accurate measures of performance breadth; and 2) more precisely model the effects of peak AS and performance breadth on latitudinal distribution. Aerobic scope reaction norms in response to temperatures may be particularly relevant for many tropical stenothermal fish species as they are reported to be the most sensitive to warming and that may be already living close to their optimum temperature for AS (Rummer et al., 2013) and have reduced thermal tolerance windows (Sunday et al., 2011, 2012). In the case of tropical stenothermal species, it would ideal to analyse species on a case by case basis given that some appear to have an adaptive capacity to adjust or shift their optimum for AS with rising temperature (Munday et al., 2016), while others seem less capable (Habary et al., 2016).

Organismal traits such as growth, reproduction and locomotion are undoubtedly relevant in species distributions and must also be considered in terms of their responses to thermal shifts. These traits might not necessarily display the same thermal reaction norm as does AS. It has also been suggested that several of these biological traits may be constrained and fail at temperatures below or above thermal limits of AS (Healy & Schulte 2012; Clark et al., 2013; Gräns et al., 2014; Norin et al., 2014 Donelson et al., 2014). Further investigations are needed to determine their role in observed species' distribution patters. Life history traits related to growth and reproductive capacity will have direct effects on the dispersal ability of species and yet for many species we still lack basic data for measures such as growth rates with age and fecundity. Without such information, it is nearly impossible to analyse to how such traits may interact with AS scope when included in models such as those performed in Chapters 2 and 3 of this thesis. At the cellular level, it has been recently proposed that the geographical distribution in fish also may be constrained by the thermal sensitivities and limits of mitochondrial stability and cardiac function (Iftikar et al., 2014). Further research is needed to confirm the generality of these findings beyond the two wrasse species that have been studied to date (Iftikar et al., 2014).

As major advances have been made in telemetry technology (Metcalfe et al., 2016b), these will likely provide major breakthroughs in our understanding of how physiological and

behavioural traits limit the distributions of species and in particular constrain the ranges of potential invaders. For example, acoustic tags are becoming much smaller than ever before with greater detection ranges and the ability to deploy multiple fish within the same aquatic system. Accelerometery technology is also rapidly advancing, which, if coupled with laboratory calibrations of oxygen consumption during activity, could be used to give information on percentage of AS a species uses under natural conditions and give a real insight into the aerobic capacity, their daily movements, and geographical range covered (Murchie et al., 2011).

### 7.8.2 The role of physiology and invasion success

Overall, invasive bullheads had a lower AS, low competitive ability for shelter, were less tolerant to hypoxia, and preferred colder temperatures compared to stone loaches. These were all contrary to expectations and suggest that these physiological and behavioural traits may have not contributed to the invasion success of bullheads in Scottish rivers. There remain a range of potential explanations for how bullheads have been able to rapidly expand their range after introduction into Scotland. For example, invasion success of bullheads might be related to human activities. Anglers are known to use bullheads as a live bait for pike fishing, possibly introducing fish to various catchments. While getting introduced into novel habitats, bullheads may have been successful in establishing high density populations compared to local stone loaches populations due to a higher reproductive output or shorter generation times (Prenda et al., 1997b). As these two fish species display a mutual avoidance behaviour, higher density numbers of bullheads in rivers might have displaced stone loaches. To confirm these speculations, field experiments need to be conducted to estimate the exact population density numbers that trigger the displacement in each of these two species.

There is still need to collect AS data on invasive fish species in particular in marine habitat as well other traits (fecundity and size/age at maturity) which can contribute to invasion success in a species. Future research should be quantifying and collecting data for these different biological parameters in order to perform models including these parameters to determine the relevance of each of them in the invasion success of species. Additionally, all these traits and complex interaction with species' AS may as well shape geographical range expansion in invasion species especially, if different biological functions are more or less important according to invasion stage (transport, introduction, establishment and spread) (Marchetti et al., 2004; García-Barthou 2007; Riberio et al., 2008).

Most research done in invasion ecology have looked into particular representative traits in invasive species in established and spreading populations. As for this specific project here, I was looking at populations of bullheads which are in the establishment and potentially in spread phase of the invasion process. I did not examine the transport an introduction phase in the case of the invasive bullhead. However, there is still a huge gap of knowledge when it comes to transport and introduction stages of the invasion process. When non-native species are introduced into a novel habitat, not all of them, or individuals within a species survive under the novel environmental conditions and thereby fail to establish. What are the underlying physiological, genetic, behavioural and other biological parameters a non-native species need to possess in order to survive the introduction phase and become an invasive species?

More research is needed to look into the differences in ecophysiological responses and interspecific interactions between other competitive pairs of native versus invasive species in order to understand the invasion success and future outcomes of these interactions in changing environmental conditions.

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# Appendix A: Publications

**A.1**: Lucon-Xiccato, T., **Nati, J.J.**, Blasco, F.R., Johansen, J.L., Steffensen, J.F. & Domenici, P. 2014 Severe hypoxia impairs lateralization in a marine teleost fish. *Journal of Experimental Biology* **217**, 4115-4118.

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SHORT COMMUNICATION

## Severe hypoxia impairs lateralization in a marine teleost fish

**Tyrone Lucon-Xiccato[1,*], Julie J. H. Nati[2], Felipe Rocco Blasco[3], Jacob L. Johansen[4], John F. Steffensen[5] and Paolo Domenici[6]**

**ABSTRACT**

In intertidal environments, the recurring hypoxic condition at low tide is one of the main factors affecting fish behaviour, causing broad effects on ecological interactions. We assessed the effects of hypoxia on lateralization (e.g. the tendency to turn left or right), a behaviour related to brain functional asymmetry, which is thought to play a key role in several life history aspects of fish. Using staghorn sculpin (*Leptocottus armatus*), a benthic fish that typically inhabits the intertidal zone, we found that hypoxia affects behavioural lateralization at the population level. On average, staghorn sculpins showed a distinct preference for right turns under normoxic conditions (>90% oxygen saturation), but an equal probability of turning right or left after exposure to hypoxia for 2 h (20% oxygen saturation). The specific turning preference observed in the staghorn sculpin control population is likely to have an adaptive value, for example in predator–prey interactions by enhancing attack success or survival from predatory attacks. Therefore the alteration of lateralization expressed by staghorn sculpins under hypoxic conditions may have far-reaching implications for species ecology and trophic interactions. Moreover, our work raises the need to study this effect in other species, in which a hypoxia-driven disruption of lateralization could affect a wider range of behaviours, such as social interactions and schooling.

**KEY WORDS: Hypoxia, Lateralization, Detour test, Staghorn sculpin**

**INTRODUCTION**

Oxygen availability is one of the main factors affecting fish physiology, behaviour and ecology. In marine intertidal environments, fish are exposed to hypoxia events daily, mainly because of tidal rhythms. Moreover, the natural occurrence of hypoxic events is exacerbated by human activities, such as agriculture and discharge of raw sewage (Diaz and Rosenberg, 2008; Richards et al., 2009). The effects of hypoxia on fish biology have been intensively studied, showing numerous interferences such as impaired performance in reproductive output, growth, swimming and schooling (Domenici et al., 2007; Richards et al., 2009).

The brain oxygen requirements are among the highest of all the organs, thus brain sensitivity to hypoxia is likely to be high. Indeed, near-lethal hypoxic conditions are known to affect brain physiology (e.g. Nilsson, 1990; Nilsson et al., 1993). Furthermore, mormyrid fish, characterized by a huge brain oxygen uptake, are known to show reduced brain size in chronic hypoxic habitats at both the inter- and intra-specific level (Chapman and Hulen, 2001). By contrast, the impact of hypoxia on behaviours directly related to brain function in fish remains relatively unexplored, although arguably, knowledge of such effects could be of great ecological relevance.

In order to assess alterations of brain functioning in hypoxic conditions in fish, we focused on behavioural lateralization, an expression of brain functional asymmetry that reflects a differential specialization of the two hemispheres in processing information (Bisazza and Brown, 2011). In fish, even though the exact evolutionary processes involved are still not clear, the lateralization patterns observed in a population are thought to reflect selective pressures and to be important for the survival of individuals in many ecological settings such as feeding, predator escape, schooling, social and sexual interactions (e.g. Bisazza et al., 1997; Bisazza et al., 2000; Dadda et al., 2010; Bisazza and Brown, 2011). Although in some cases hemispheric specialization could represent an added cost in information processing (Bisazza and Brown, 2011), the vast of majority of evidence suggests that it confers several advantages. A lateralized brain allows a separation of task achievements: each hemisphere can develop higher specialization in processing specific information, resulting in greater cognitive performance. For example, highly lateralized individual fish (i.e. those with greater individual-level lateralization) outperform non-lateralized ones in spatial reorientation tasks or in simultaneous activities, such as foraging and predator vigilance (Sovrano et al., 2005; Dadda and Bisazza, 2006). Moreover, lateralization is also associated with an enhanced ability to escape from a predator: strongly lateralized fish have a faster reaction time, higher turning rates and longer distance travelled in escape responses (Dadda et al., 2010). In some cases, a bias for a specific directionality of lateralization is observed at the population level: a conspicuous proportion of individuals in a population is lateralized in the same direction (Bisazza and Brown, 2011). This bias appears to be relevant in social species to achieve greater coordination in movements (Bisazza et al., 2000), whereas in non-gregarious species, it could be involved in prey–predator interactions (Yasugi and Hori, 2012).

In this experiment, we assessed the effect of hypoxia on behavioural lateralization in the staghorn sculpin (*Leptocottus armatus* Girard 1854), a benthic fish that typically inhabits the intertidal environment and may therefore experience low oxygen availability (<20% oxygen saturation) during stranding in mudflats and tidepools (Palsson et al., 2008). We exposed two groups of staghorn sculpins to normoxia (>90% oxygen saturation) and non-lethal levels of hypoxia that naturally occur in the sampling area (20% oxygen saturation) (Palsson et al., 2008; Speers-Roesch et al., 2013), respectively, and we observed the subjects in a turning preference test (detour test) to evaluate their behavioural lateralization. We then compared the population- and individual-

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The Journal of Experimental Biology

**A.2** Killen, S.S., **Nati, J.J**. & Suski, C.D. 2015 Vulnerability of individual fish to capture by trawling is influenced by capacity for anaerobic metabolism. *Proceedings Biological Sciences / The Royal Society* **282**, 20150603.

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THE ROYAL SOCIETY PUBLISHING

# Vulnerability of individual fish to capture by trawling is influenced by capacity for anaerobic metabolism

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The harvest of animals by humans may constitute one of the strongest evolutionary forces affecting wild populations. Vulnerability to harvest varies among individuals within species according to behavioural phenotypes, but we lack fundamental information regarding the physiological mechanisms underlying harvest-induced selection. It is unknown, for example, what physiological traits make some individual fish more susceptible to capture by commercial fisheries. Active fishing methods such as trawling pursue fish during harvest attempts, causing fish to use both aerobic steady-state swimming and anaerobic burst-type swimming to evade capture. Using simulated trawling procedures with schools of wild minnows *Phoxinus phoxinus*, we investigate two key questions to the study of fisheries-induced evolution that have been impossible to address using large-scale trawls: (i) are some individuals within a fish shoal consistently more susceptible to capture by trawling than others?; and (ii) if so, is this related to individual differences in swimming performance and metabolism? Results provide the first evidence of repeatable variation in susceptibility to trawling that is strongly related to anaerobic capacity and swimming ability. Maximum aerobic swim speed was also negatively correlated with vulnerability to trawling. Standard metabolic rate was highest among fish that were least vulnerable to trawling, but this relationship probably arose through correlations with anaerobic capacity. These results indicate that vulnerability to trawling is linked to anaerobic swimming performance and metabolic demand, drawing parallels with factors influencing susceptibility to natural predators. Selection on these traits by fisheries could induce shifts in the fundamental physiological makeup and function of descendent populations.

## 1. Introduction

Humans are effective predators, and selective harvest of animals by humans probably represents one of the strongest drivers of evolutionary change for wild animal populations [1–3]. Hunting and fishing are selective processes which often remove individuals that, under normal circumstances, may have the highest reproductive potential [2,4,5]. Available evidence suggests that selection by harvest can lead to genetic change within wild populations for specific traits [1,5–7]. For example, the preferential harvest of large individuals from fish populations has caused evolutionary changes to key life-history traits, including growth trajectories, reproductive investment and size at maturity [8–11], a phenomenon termed 'fisheries-induced evolution' (FIE) [8,12–14]. In addition to varying in their *desirability* as a target for harvest, however, individual animals also differ in their *vulnerability* to harvest [13,15,16]. Interestingly, artificial selection on traits that affect harvest vulnerability can sometimes act in parallel with natural selection when selecting on specific traits. For example, several recent studies indicate that individual variation in behaviour can influence

**A.3 Nati, J.J.H.**, Lindström, J., Halsey, L.G. & Killen, S.S. 2016 Is there a trade-off between peak performance and performance breadth across temperatures for aerobic scope in teleost fishes?. *Biology Letters*.

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THE ROYAL SOCIETY PUBLISHING

Physiology

# Is there a trade-off between peak performance and performance breadth across temperatures for aerobic scope in teleost fishes?

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The physiology and behaviour of ectotherms are strongly influenced by environmental temperature. A general hypothesis is that for performance traits, such as those related to growth, metabolism or locomotion, species face a trade-off between being a thermal specialist or a thermal generalist, implying a negative correlation between peak performance and performance breadth across a range of temperatures. Focusing on teleost fishes, we performed a phylogenetically informed comparative analysis of the relationship between performance peak and breadth for aerobic scope (AS), which represents whole-animal capacity available to carry out simultaneous oxygen-demanding processes (e.g. growth, locomotion, reproduction) above maintenance. Literature data for 28 species indicate that peak aerobic capacity is not linked to thermal performance breadth and that other physiological factors affecting thermal tolerance may prevent such a trade-off from emerging. The results therefore suggest that functional links between peak and thermal breadth for AS may not constrain evolutionary responses to environmental changes such as climate warming.

## 1. Introduction

For ectotherms, performance traits related to growth, reproduction and locomotion are often depicted using thermal performance curves [1,2] that illustrate how a trait responds to variation in environmental temperature (figure 1). Evolutionary thermal adaptation [2] may result in thermal specialists or thermal generalists, performing better over a narrow versus a broad range of temperatures, respectively (figure 1; [3]). Thermal and biochemical constraints on enzyme structure and function and membrane fluidity suggest that adaptations for increased performance at one temperature may cause decreased performance at other temperatures, resulting in a trade-off between peak performance ($P_{max}$) at a thermal optimum and thermal performance breadth ($T_{breadth}$). Owing to these potential compromises, previous researchers have suggested that a 'jack of all temperatures is a master of none' [4].

While a trade-off between $P_{max}$ and $T_{breadth}$ is predicted by theory [3,5], several studies have documented that an increased performance capacity at one temperature does not necessarily lead to reduced performance at other temperatures [4,6–9]. Notably, however, most studies have examined the differences in performance among populations of the same species, with few attempts to examine whether a trade-off exists across species. Therefore, it remains unknown whether any trade-off between $P_{max}$ and $T_{breadth}$ generate interspecific constraints on thermal

# References

Abdoli, A., Pont, D. & Sagnes, P. 2007 Intrabasin variations in age and growth of bullhead: the effects of temperature. *Journal of Fish Biology* **70**, 1224–1238. (doi:10.1111/j.1095-8649.2007.01400.x).

Addo-Bediako, A., Chown, S.L. & Gaston, K.J. 2000 Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B: Biological Sciences* **267**, 739–45.

Alcaraz, C., Bisazza, A. & García-Berthou, E. 2008 Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia* **155**, 205–213. (doi:10.1007/s00442-007-0899-4).

Amarasekare, P. & Coutinho, R.M. 2014 Effects of temperature on intraspecific competition in ectotherms. *The American Naturalist* **184**, 50–65. (doi:10.1086/677386).

Anderson, H., Futter, M., Oliver, I., Redshaw, J. & Harper, A. 2010 Trends in Scottish river water quality.

Andrewartha, H.G. & Birch, L.C. 1954 The distribution and abundance of animals. University of Chicago Press, Chicago.

Angilletta, M.J. 2006 Estimating and comparing thermal performance curves. *Journal of Thermal Biology* **31**, 541-545.

Angilletta, M.J. 2009 Thermal Adaptation: A Theoretical and Empirical Synthesis. (Oxford, Oxford University Press).

Angilletta, M.J., Huey, R.B. & Frazier, M.R. 2010 Thermodynamic Effects on Organismal Performance: Is Hotter Better? *Physiological and Biochemical Zoology* **83**, 197-206. (doi:10.1086/648567).

Bates, A.E., McKelvie, C.M., Sorte, C.J.B., Morley, S.A., Jones, N.A.R., Mondon, J.A., et al. 2013 Geographical range, heat tolerance and invasion success in aquatic species. *Proceedings of the Royal Society of London B: Biological Sciences* **280**, 1-7. (doi:ARTN 20131958\r10.1098/rspb.2013.1958).

Bartoń, K. 2015 MuMIn: Multi-Model Inference. In *R package version 1.15.1*.

Becker, J., Ortmann, C., Wetzel, M.A., & Koop, J.H.E. 2016 Metabolic activity and behavior of the invasive amphipod Dikerogammarus villosus and two common Central European gammarid species (Gammarus fossarum, Gammarus roeselii): Low metabolic rates may favor the invader. *Comparative Biochemistry and Physiology -Part A: Molecular and Integrative Physiology* **191**, 119–126. (doi:10.1016/j.cbpa.2015.10.015).

Berger, D., Walters, R. & Gotthard, K. 2008 What limits insect fecundity? Body size- and temperature-dependent egg maturation and oviposition in a butterfly. *Functional Ecology* **22**, 523–529. (doi :10.1111/j.1365-2435.2008.01392.x).

Bennett, A.F. 1990 Thermal dependence of locomotor capacity. *The American Physiological Society* R253–R258.

Biro, P. & Stamps J. 2010 Do Consistent Individual Differences in Metabolic Rate Promote Consistent Individual Differences in Behavior? *Trends in Ecology & Evolution* **25**, 653–659.

Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošík, V. et al. 2011 A proposed unified framework for biological invasions. *Trends in Ecology & Evolution* **26** (**7**), 333–339. (doi:10.1016/j.tree.2011.03.023).

Blanchet, S., Loot, G., Grenouillet, G. & Brosse, S. 2007 Competitive interactions between native and exotic salmonids: a combined field and laboratory demonstration. *Ecology of Freshwater Fish* **16,** 133–143.
Bohlen, J. 2003 Temperature and oxygen requirements of early life stages of the endangered spined loach, *Cobitis taenia* L. (Teleostei, Cobitidae) with implications for the management of natural populations. *Archiv für Hydrobiologie* **157**, 195-212.

Brady, D.C., Targett, T.E. & Tuzzolino, D.M. 2009 Behavioral responses of juvenile weakfish (*Cynoscion regalis*) to diel-cycling hypoxia: swimming speed, angular correlation, expected displacement, and effects of hypoxia acclimation. *Canadian Journal of Fisheries and Aquatic Sciences* **66**, 415–424.

Brown, L.R. & Moyle, P.B. 1991 Changes in habitat and microhabitat partitioning within an assemblage of stream fishes in response to predation by Sacramento squawfish (Ptychocheilus grandis). *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 849–856. (doi:10.1139/f91-101).

Brett, J.R., Shelbourn, J.E. & Shoop, C.T. 1969 Growth Rate and Body Cornposition of Fingerling Sockeye Salmon, Oncorhynchus nerkao in relation to Ternperature and Ration Size. *Journal of the Fisheries Research Board of Canada* **26**, 2363–2394. (doi :10.1139/f69-229).

Britton, J.R., Cucherousset, J., Davies, G.D., Godard, M.J. & Copp, G.H. 2010 Non-native fishes and climate change: predicting species responses to warming temperatures in a temperate region. *Freshwater Biology* **55**, 1130–1141.

Bozinovic, F., Calosi, P. & Spicer, J.I. 2011 Physiological Correlates of Geographic Range in Animals. *Annual Review of Ecology, Evolution, and Systematics* **42**, 155–179. (doi: 10.1146/annurev-ecolsys-102710-145055).

Briffa, M. & Sneddon, L.U. 2007 Physiological constraints on contest behaviour. *Functional Ecology* **21**, 627–637. (doi:10.1111/j.1365-2435.2006.01188.x).

Broughton R.E., Betancur-R.R., Li C., Arratia G., Ortí G. 2013 Multi-locus phylogenetic analysis reveals the pattern and tempo of bony fish evolution. *PLOS Currents Tree of Life*. (doi: 10.1371/currents.tol.2ca8041495ffafd0c92756e75247483e.).

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. 2004 Toward a metabolic theory of ecology. *Ecology* **85**, 1771-1789.

Brown, J.H. & Sax, D.O.V.F. 2005 Short Note Biological invasions and scientific objectivity: Reply to Cassey. *Austral Ecology* **30**, 481–483.

Callaway, R.M. & Ridenour, W.M. 2004 Novel weapons: Invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* **2**, 436–443. (doi:10.1890/1540-9295).

Calosi, P., Bilton, D.T., Spicer, J.I., Votier, S.C. & Atfield, A. 2010 What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). *Journal of Animal Ecology* **79**,194–204.

Careau, V., Thomas, D., Humphries, M.M. & Re, D. 2008 Energy metabolism and animal personality. *Oikos* **117**, 641–653. (doi:10.1111/j.2008.0030-1299.16513.x).

Carmona-Catot, G., Magellan, K. & García-Berthou, E. 2013 Temperature-Specific Competition between Invasive Mosquitofish and an Endangered Cyprinodontid Fish. *PloS one* **8**, 1-7.

Chabot, D. & Claireaux, G. 2008 Environmental hypoxia as a metabolic constraint on fish: the case of Atlantic cod, *Gadus morhua*. *Marine Pollution Bulletin* **57**, 287–294. (doi: 10.1016/j.marpolbul.2008.04.001).

Chamberlain, S.A., Bronstein, J.L. & Rudgers, J.A. 2014 How context dependent are species interactions? *Ecology Letters* **17**, 881–890. (doi:10.1111/ele.12279).

Chen, I., Hill, J.K., Ohlemüller, R., Roy, D.B. & Thomas, C.D. 2011 Rapid range shifts of species of climate warming. *Science* **333**, 1024–1026. (doi:10.1126/science.1206432).

Claireaux, G. & Lefrançois, C. 2007 Linking environmental variability and fish performance: integration through the concept of scope for activity. *Proceedings of the Royal Society of London B: Biological Sciences* **362**, 2031–2041. (doi:10.1098/rstb.2007.2099).

Clark, T.D., Sandblom, E. & Jutfelt, F. 2013 Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *The Journal of Experimental Biology* **216**, 2771-2782. (doi:10.1242/jeb.084251).

Cook, D.G. & Herbert, N.A. 2012a Low O2 avoidance is associated with physiological perturbation but not exhaustion in the snapper (*Pagrus auratus*: Sparidae). *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **162**, 310–316. (doi:10.1016/j.cbpa.2012.03.024).

Cook, D.G.& Herbert, N.A. 2012b The physiological and behavioural response of juvenile kingfish (*Seriola lalandi*) differs between escapable and inescapable progressive hypoxia. *Journal of Experimental Marine Biology and Ecology* **413**, 138–144. (doi:10.1016/j.jembe.2011.12.006).

Cook, D.G., Iftikar, F.I., Baker, D.W., Hickey, A.J.R. & Herbert, N.A. 2013 Low-$O_2$ acclimation shifts the hypoxia avoidance behaviour of snapper (*Pagrus auratus*) with only subtle changes in aerobic and anaerobic function. *The Journal of Experimental Biology* **216**, 369–378. (doi:10.1242/jeb.073023).

Cooke, S.J., Sack, L., Franklin, C.E., Farrell, A.P., Beardall, J., Wikelski, M. & Chown, S. L. 2013 What is conservation physiology? Perspectives on an increasingly integrated and essential science. *Conservation Physiology* **1**, 1–23. (doi :10.1093/conphys/cot001).

Copp, G., Bianco, P., Bogutskaya, N.G., Erös, T., Falka, I., Ferreira, M.T. et al. 2005 To be, or not to be a non-native freshwater fish? *Journal of Applied Ichthyology* **21**, 242–262. (doi:10.1111/j.1439-0426.2005.00690.x).

Cossins, A. & Prosser, C. 1978 Evolutionary adaptation of membranes to temperature. *Proceedings of the National Academy of Sciences* **75**, 2040-2043.

Costanzo, K.I., Kesavaraju, B. & Juliano, S.A. 2005 Condition-specific competition in container mosquitoes: the role of noncompeting life-history stages. *Ecology* **86**, 3289–3295.

Costedoat, C., Pech, N., Chappaz, R., Salducci, M.D., Lim, P. & Gilles, A. 2004 Study of introgressive hybridization between Chondrostoma t. toxostoma and Chondrostoma n. nasus (Teleostei, Cyprinidae) using multiple approaches. *Cybium* **28**, 51–61.

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T. & Sih, A. 2010 Personality traits and dispersal tendency in the invasive mosquitofish (Gambusia affinis). *Proceedings of Biological sciences / The Royal Society* **277**, 1571–1579.

Coutant, C.C. 1977 Compilation of Temperature Preference Data. *Journal of the Fisheries Research Board of Canada* **34**, 739–745. (doi:10.1139/f77-115).

Cutts, C.J., Metcalfe, N.B. & Taylor, A.C. 1998 Aggression and growth depression in juvenile salmon – the consequences of variation in metabolic rate. *Journal of Fish Biology* **52**, 1026–1037.

D'Amato, M.E., Esterhuyse, M.M., van derWaal, B.C.W., Brink, D. & Volckaert, F.A.M. 2007 Hybridization and phylogeography of the Mozambique tilapia *Oreochromis mossambicus* in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. *Conservation Genetics* **8,** 475–488.

Davey, A.J.H., Hawkins, S.J., Turner, G.F. & Doncaster, C.P. 2005 Size-dependent microhabitat use and intraspecific competition in Cottus gobio. *Journal of Fish Biology* **67**, 428–443. (doi:10.1111/j.0022-1112.2005.00736.x)

de Gelder, S., van der Velde, G., Platvoet, D., Leung, N., Dorenbosch, M., Hendriks, H.W.M. & Leuven, R.S.E.W. 2016 Competition for shelter sites: Testing a possible mechanism for gammarid species displacements. *Basic and Applied Ecology* 1–8. (doi:10.1016/j.baae.2016.01.008).

Demer, D.A., Zwolinski, J.P., Byers, K.A., Cutter, G.R., Renfree, J.S., Sessions, T.S. & Macewicz, B.J. 2012 Prediction and confirmation of seasonal migration of Pacific sardine (Sardinops sagax) in the California Current Ecosystem. *Fishery Bulletin* **110**, 52–70.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. & Martin, P.R. 2008 Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences* **105**, 6668–6672. (doi:10.1073/pnas.0709472105).

Diaz, R.J. 2001 Overview of hypoxia around the world. *Journal of Environmental Quality* **30**(**2**), 275–281.

Diaz, R.J. & Rosenberg, R. 2008 Spreading dead zones and consequences for marine ecosystems. *Science* **321**, 926–929. (doi:10.1126/science.1156401).

Dillon, M.E., Wang, G. & Huey, R.B. 2010 Global metabolic impacts of recent climate warming. *Nature* **467**, 704–706. (doi:10.1038/nature09407).

Domenici, P., Steffensen, J.F & Batty, R.S. 2000 The effect of progressive hypoxia on swimming activity and schooling in Atlantic herring. *Journal of Fish Biology* **57**, 1526–1538. (doi:10.1006/jfbi.2000.1413).

Domenici, P., Lefrançois, C. & Shingles, A. 2007 Hypoxia and the antipredator behaviours of fishes. *Philosophical transactions of the Royal Society of London. Series B, Biological scienc*es **362**, 2105–2121. (doi:10.1098/rstb.2007.2103).

Donelson, J.M., McCormick, M.I., Booth, D.J., Munday, P.L. 2014 Reproductive acclimation to increased water temperature in a tropical reef fish. *PloS one* **9**, e97223. (doi:10.1371/journal.pone.00972).

Downhower, J.F., Lejeune, P., Gaudin, P. & Brown, L. 1990 Movements of the chabot (Cottus gobio) in a small stream. Polskie *Archiwum Hydrobiologii* **37**,119–126.

Dubs, D.O.L. & Corkum, L.D. 1996 Behavioral interactions between round gobies (Neogobius melanostomus) and Mottled Sculpins (Cottus bairdi). *Journal of Great Lakes Research* **22**, 838-844. (doi:10.1016/S0380-1330(96)71005-5).

Dupont-Prinet, A., Chatain, B., Grima, L., Vandeputte, M., Claireaux, G. & McKenzie, D.J. 2010 Physiological mechanisms underlying a trade-off between growth rate and tolerance of feed deprivation in the European sea bass (Dicentrarchus labrax). *Journal of Experimental Biology* **113**, 1143-1152.

Dupont-Prinet, A., Vagner, M., Chabot D. & Audet C. 2013 Impact of hypoxia on the metabolism of Greenland halibut (*Reinhardtius hippoglossoides*). *Canadian Journal of Fisheries and Aquatic Sciences* **70**, 461–469. (doi:10.1139/cjfas-2012-0327).

Elshout, P.M.F., Dionisio Pires, L. M., Leuven, R.S.E.W., Wendelaar Bonga, S.E. & Hendriks, A.J. 2013 Low oxygen tolerance of different life stages of temperate freshwater fish species. *Journal of Fish Biology* **83**, 190–206. (doi:10.1111/jfb.12167).

Ehrlich P.R. 1986 Which animal will invade? In: Mooney HA and Drake JA (eds) Ecology of Biological Invasions of North American and Hawaii, pp 79–95. Springer-Verlag, New York.

Elgar, M.A. 1990 Evolutionary Compromise between a Few Large and Many Small Eggs: Comparative Evidence in Teleost Fish. *Oikos* **59**, 283–287.

Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K. M. et al. 2011 Differences in thermal tolerance among sockeye salmon populations. *Science* **332**, 109–112. (doi:10.1126/science.11991508).

Elliott, J.M., Elliott, J.A. & Allonby, J.D. 1994 The critical thermal limits for the stone loach, Noemacheilus harhatulus , from three populations in north-west England. *Freshwater Biology* **32**, 593–601.

Elliott, J. & Elliott, J. 1995 The critical thermal limits for the bullhead, Cottus gobio, from three populations in north-west England. *Freshwater Biology* **33**, 411–419. (doi:10.1111/j.1365-2427.1994.tb01150.x)

Elliott, J.M., Hurley, M.A. & Allonby, J.D. 1996 A functional model for maximum growth of immature stone-loach, Barbatula barbatula, from three popula- tions in north-west England. *Freshwater Biology* **36**, 547–554.

Evans, D.O. 2007 Effects of hypoxia on scope-for-activity and power capacity of lake trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 345–361. (doi:10.1139/F07-007).

Fausch, K.D. 1988 Tests of Competition between Native and Introduced Salmonids in Streams: What Have We Learned? *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 2238–2246. (doi:10.1139/f88-260).

Farrell, A.P. 2013 Aerobic scope and its optimum temperature: clarifying their usefulness and limitations - correspondence on J. Exp. Biol. 216, 2771-2782. *Journal of Experimental Biology* **216**, 4493-4494.

Farrell, A.P. 2016 Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *Journal of Fish Biology* **88**, 322-343. (doi:10.1111/jfb.12789).

Fatio, V. 1882 Faune des vertèbres de la Suisse. 4. Poissons I. Genbve et Bale.

Feary, D.A, Pratchett, M.S., J Emslie, M., Fowler, A.M., Figueira, W.F., Luiz, et al. 2013 Latitudinal shifts in coral reef fishes: why some species do and others do not shift. *Fish and Fisheries*, 1–23. (doi.org/10.1111/faf.12036).

Ficke, A.D., Myrick, C.A. & Hansen, L.J. 2007 Potential impacts of global climate change on freshwater fisheries. *Reviews in Fish Biology and Fisheries* **17**, 581–613.

Fincke, O.M. 1992 Interspecific Competition for Tree Holes: Consequences for Mating Systems and Coexistence in Neotropical Damselflies. *The American Naturalist* **139**, 80–101.

Fischer, P. 2004 Noctural foraging in the stone loach (Barbatula barbatula): Fixed or environmentally mediated behaviour? *Journal of Freshwater Ecology* **19**. (doi: 10.1080/02705060.2004.9664515).

Fobert, E., Fox, M. G., Ridgway, M. & Copp, G. H. 2011 Heated competition: how climate change will affect non-native pumpkinseed Lepomis gibbosus and native perch Perca fluviatilis interactions in the U.K. *Journal of Fish Biology* **79**, 1592–1607. (doi:10.1111/j.1095-8649.2011.03083.x).

Freckleton, R.P., Harvey, P.H. & Pagel, M. 2002 Phylogenetic analysis and comparative data: a test and review of evidence. *The American Naturalist* **160**, 712-726.

Frisk, M., Skov, P.V. & Steffensen, J.F. 2012 Thermal optimum for pikeperch (Sander lucioperca) and the use of ventilation frequency as a predictor of metabolic rate. *Aquaculture* **324**, 151-157.

Freitas, C., Olsen, E.M., Knutsen, H., Albretsen, J. & Moland, E. 2016 Temperature-associated habitat selection in a cold-water marine fish. *Journal of Animal Ecology* **85**, 628–637. (doi:10.1111/1365-2656.12458).

Froese, R. & Pauly D. Editors. 2014. FishBase. World Wide Web electronic publication. www.fishbase.org, version (06/2014).

Fry, F.E.J. 1947 Effect of the environment on animal activity. *Univ. Toronto Stud. Biol. Ser*. **55**, 1–62.

Fry, F.E.J. 1971 The Effect of Environmental Factors on the Physiology of Fish. In *Fish Physiology* (pp. 1-98), Academic Press.

Fu, S.-J., Zeng, L.-Q., Li, X.-M., Pang, X. et al. 2009 The behavioural, digestive and metabolic characteristics of fishes with different foraging strategies. *The Journal of Experimental Biology* **212**, 2296–2302.

Gardiner, N.M., Munday, P.L. & Nilsson, G.E. 2010 Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PloS one* **5**, 1-13. (doi:10.1371/journal.pone.0013299).

García-Berthou, E., Alcaraz, C., Pou-Rovira, Q., Zamora, L., Coenders, G. & Feo, C. 2005 Introduction pathways and establishment rates of invasive aquatic species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 453–463. (doi:10.1139/f05-017).

García-Berthou, E. 2007 The characteristics of invasive fishes: What has been learned so far? *Journal of Fish Biology* **71**, 33–55. (doi:10.1111/j.1095-8649.2007.01514.x).

Garland Jr, T. & Ives, A.R. 2000 Using the past to predict the present: confidence intervals for regression equations in phylogenetic comparative methods. *The American Naturalist* **155**, 346-364.

Gaston, K.J. 2003 The structure and dynamics of geographic ranges. Oxford, UK: Oxford University Press.

Gaston, K.J. 2009 Geographic Range Limits of Species. *Proceedings of Biological sciences / The Royal Society* **276**, 1391–1393. (doi:10.1098/rspb.2009.0100).

Gaston, K.J., Chown, S.L., Calosi, P., Bernardo, J., Bilton, D.T., Clarke, A., et al. 2009 Macrophysiology: a conceptual reunification. *The American Naturalist*, **174**, 595–612. (doi:/10.1086/605982).

Gilchrist, G.W. 1995 Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. *The American Naturalist*, 252-270.

Gozlan, R.E., St-Hilaire, S., Feist, S.W., Martin, P. & Kent, M.L. 2005 Biodiversity disease threat to European fish. *Nature* **435,** 1046.

Gozlan, R.E., Peeler, E.J., Longshaw, M., St-Hilaire, S. & Feist, S.W. 2006 Effect of microbial pathogens on the diversity of aquatic populations, notably in Europe. *Microbes and Infection* **8**, 1358–1364. (doi:10.1016/j.micinf.2005.12.010).

Gozlan, R.E. 2008 Introduction of non-native freshwater fish: is it all bad? *Fish and Fisheries* **9,** 106–115.

Gozlan, R.E. & Newton, A.C. 2009 Biological invasions: benefits versus risks. *Science* **324**, 1015–1016.

Grabowska, J., Kakareko, T., Błońska, D., Przybylski, M., Kobak, J., Jermacz & Copp, G. H. 2016 Interspecific competition for a shelter between non-native racer goby and native European bullhead under experimental conditions - Effects of season, fish size and light conditions. *Limnologica* **56**, 30–38. (doi:10.1016/j.limno.2015.11.004).

Grafen, A. 1989 The phylogenetic regression. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **326**, 119-157.

Graham, C.T. & Harrod, C. 2009 Implications of climate change for the fishes of the British Isles. *Journal of Fish Biology* **74**, 1143–1205.

Gräns, A., Jutfelt, F., Sandblom, E., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O., Einarsdottir, I. et al. 2014 Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO2 in Atlantic halibut. *Journal of Experimental Biology* **217**, 711-717.

Habary, A., Johansen, J.L., Nay, T.J., Steffensen, J.F., & Rummer, J.L. 2016 Adapt, move or die - how will tropical coral reef fishes cope with ocean warming? *Global Change Biology*. (doi:10.1111/gcb.13488).

Hack, M.A. 1997 The energetic costs of fighting in the house cricket, Acheta domesticus L. *Behavioral Ecology* **8**, 28–36. (doi:10.1093/beheco/8.1.28).

He, W., Cao, Z.D. & Fu, S.J. 2015 Effect of temperature on hypoxia tolerance and its underlying biochemical mechanism in two juvenile cyprinids exhibiting distinct hypoxia sensitivities. *Comparative Biochemistry and Physiology -Part A: Molecular and Integrative Physiology* **187**, 232–241. (doi:10.1016/j.cbpa.2014.05.004)

Healy, T.M. & Schulte, P.M. 2012 Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish (Fundulus heteroclitus). *Physiological and Biochemical Zoology* **85**, 107-119.

Herbert, N.A. & Steffensen, J.F. 2005 The response of Atlantic cod, *Gadus morhua*, to progressive hypoxia: fish swimming speed and physiological stress. *Marine Biology* **147**, 1403–1412. (doi:10.1007/s00227-005-0003-8).

Herbert, N.A., Skjæraasen, J.E., Nilsen, T., Salvanes, A.G.V. & Steffensen, J.F. 2011 The hypoxia avoidance behaviour of juvenile Atlantic cod (*Gadus morhua* L.) depends on the provision and pressure level of an O2 refuge. *Marine Biology* **158**, 737–746. (doi:10.1007/s00227-010-1601-7).

Hinchliff, C.E., Smith, S.A., Allman, J.F., Burleigh, J.G., Chaudhary, R., Coghill, L.M., Crandall, K.A., Deng, J., Drew, B.T. & Gazis, R. 2015 Synthesis of phylogeny and

taxonomy into a comprehensive tree of life. *Proceedings of the National Academy of Sciences* **112**, 12764-12769.

Huntingford, F., Taylor, A., Smith, I. & Thorpe, K. 1995 Behavioural and physiological studies of aggression in swimming crabs. *Journal of Experimental Marine Biology and Ecology* **193**, 21–39. (doi:10.1016/0022-0981(95)00108-5).

Holway, D.A., Suarez, A.V. & Case, T.J. 2002 Role of abiotic factors in governing susceptibility to invasion: a test with argentine ants. *Ecology* **83**, 1610–1619.

Houde, A.L.S., Wilson, C.C. & Neff, B.D. 2015 Competitive interactions among multiple non-native salmonids and two populations of Atlantic salmon. *Ecology of Freshwater Fish* **24**, 44–55. (doi:10.1111/eff.12123).

Huey, R.B. & Stevenson, R. 1979 Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *American Zoologist* **19**, 357-366.

Huey, R.B. & Hertz, P.E. 1984 Is a jack-of-all-temperatures a master of none? *Evolution* **38**, 441-444.

Huey, R.B.,Deutsch, C.A.,Tewksbury, J.J., Vitt, L.J., Hertz, P.E. et al. 2009 Why tropical forest lizards are vulnerable to climate warming. *Proceedings of Biological sciences / The Royal Society 276*, 1939–1948

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M. & Williams, S.E. 2012 Predicting Organismal Vulnerability to Climate Warming: Roles of Behaviour, Physiology and Adaptation. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **367**,1665–1679. (doi:10.1098/rstb.2012.0005).

Iftikar, F.I & Hickey, A.J.R. 2013 Do Mitochondria Limit Hot Fish Hearts? Understanding the Role of Mitochondrial Function with Heat Stress in Notolabrus celidotus. *PLoS one* **8**, e64120. (doi:10.1371/journal.pone.0064120).

Iftikar, F.I., MacDonald, J.R., Baker, D.W., Renshaw, G.M.C. & Hickey, A.J.R. 2014 Could thermal sensitivity of mitochondria determine species distribution in a changing climate? *Journal of Experimental Biology* **217**, 2348–2357. (doi:10.1242/jeb.098798).

Janzen, D.H. 1967 Why mountain passes are higher in the tropics. *The American Naturalist* **101,** 233–249.

Jewett, E.B., Hines, A.H., Ruiz, G.M., 2005 Epifaunal disturbance by periodic low levels of dissolved oxygen: native vs. invasive species response. *Marine Ecology Progress Series* **304**, 31–44.

Jobling, M. 1981 Temperature tolerance and the final preferendum-rapid methods for the assessment of optimum growth temperatures. *Journal of Fish Biology* **19**, 439–455. (doi:10.1111/j.1095-8649.1981.tb05847.x).

Kahn, J.R. & Herbert, N.A. 2012 The behavioural thermal preference of the common triplefin (Forsterygion lapillum) tracks aerobic scope optima at the upper thermal limit of its distribution. *Journal of Thermal Biology* **37**, 118–124. (doi:10.1016/j.jtherbio.2011.11.009).

Kakareko, T., Kobak, J., Poznańska, M., Jermacz, L. & Copp, G. H. 2014 Underwater evaluation of habitat partitioning in a European river between a non-native invader, the racer goby and a threatened native fish, the European bullhead. *Ecology of Freshwater Fish* **25**, 60–71. (doi:10.1111/eff.12191).

Knaepkens, G., Bruyndoncx, L. & Eens, M. 2004 Assessment of residency and movement of the endangered bullhead (Cottus gobio) in two Flemish rivers. *Ecology of Freshwater Fish* **13**, 317–322. (doi:10.1111/j.1600-0633.2004.00065.x).

Kearney, M. & Porter, W. 2009 Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology Letters* **12**, 334–350. (doi:10.1111/j.1461-0248.2008.01277.x).

Kelley, A.L. 2014 The role thermal physiology plays in species invasion. *Conservation Physiology* **2**, 1–14. (doi:10.1093/conphys/cou045).

Killen, S.S, Costa I., Brown J. & Gamperl K. 2007 Little Left in the Tank: Metabolic Scaling in Marine Teleosts and Its Implications for Aerobic Scope. *Proceedings Biological Sciences / The Royal Society* **274**, 431–438.

Killen, S.S., Atkinson, D. & Glazier, D.S. 2010 The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecology Letters* **13**, 184–193. (doi:10.1111/j.1461-0248.2009.01415.x).

Killen, S.S., Marras, S., Steffensen, J.F & McKenzie, D.J. 2012 Aerobic capacity influences the spatial position of individuals within fish schools. *Proceedings of the Royal Society B: Biological Sciences* **279**, 357-364.

Killen, S.S., Marras, S., Metcalfe, N.B, McKenzie, D.J & Domenici, P. 2013 Environmental Stressors Alter Relationships Between Physiology and Behaviour. *Trends in Ecology & Evolution* **1702**, 1–8.

Killen, S. S., Mitchell, M.D., Rummer, J.L., Chivers, D.P., Ferrari, M.C.O., Meekan, M.G., & McCormick, M.I. 2014 Aerobic Scope Predicts Dominance During Early Life in a Tropical Damselfish. *Functional Ecology* **28**, 1367–1376.

Killen, S. S. 2014 Growth Trajectory Influences Temperature Preference in Fish Through an Effect on Metabolic Rate. *The Journal of Animal Ecology* **83**, 1-10.

Killen, S.S., Nati, J.J.H., & Suski, C.D. 2015 Vulnerability of individual fish to capture by trawling is influenced by capacity for anaerobic metabolism. *Proceedings Biological Sciences / The Royal Society* **282**, 1-7. (doi:10.1098/rspb.2015.0603).

Killen, S.S., Glazier, D., Rezende, E.L., Clark, T., Atkinson, D., Willener, A. & Halsey, L.G. 2016 Ecological influences and physiological correlates of metabolic rates in teleost fishes. *The American Naturalist* **187**, 592-606.

Kita, J., Tsuchida, S. & Setoguma, T. 1996 Temperature preference and tolerance, and oxygen consumption of the marbled rockfish, Sebastiscus marmoratus. *Marine Biology* **125**, 467. (doi:10.1007/BF00353259).

Kitchell, J.F., Schindler, D.E., Ogutu-Ohwayo, R. & Reinthal, P.N. 1997 The Nile perch in Lake Victoria: interactions between predation and fisheries. *Ecological Applications* **7,** 653–664.

Knaepkens, G., Bruyndoncx, L. & Eens, M. 2004 Assessment of Residency and Movement of the Endangered Bullhead (*Cottus Gobio*) in Two Flemish Rivers." *Ecology of Freshwater Fish* **13** (**4**), 317–322. (doi:10.1111/j.1600-0633.2004.00065.x).

Kolar, C.S. & Lodge, D.M. 2002 Ecological predictions and risk assessment for alien fishes in North America. *Science* **298**, 1233–1236. (doi:10.1126/science.1075753).

Kubisch, A., Holt, R., Poethke, H. & Fronhofer, E. 2013 Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. *Oikos* **000**, 1–18. (doi:10.1111/j.1600-0706.2013.00706.x).

Kuznetsova A., Brockhoff P.B., Bojesen R.H. 2016 Package "lmerTest" in R. Tests in Linear Mixed Effects Models.

Landman, M.J., Van Den Heuvel, M.R. & Ling, N. 2005 Relative sensitivities of common freshwater fish and invertebrates to acute hypoxia. *New Zealand Journal of Marine and Freshwater Research* **39**, 1061–1067. (doi:10.1080/00288330.2005.9517375).

Lefevre, S., Huong D., T., T., Wang T., Phuong, N., T. & Bayley M. 2011 Hypoxia tolerance and partitioning of bimodal respiration in the striped catfish (Pangasianodon hypophthalmus). *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* **158**(**2**), 207–214.

Lefevre, S., Damsgaard, C., Pascale, D.R., Nilsson, G.E. & Stecyk, J.A.W. 2014 Air breathing in the Arctic: influence of temperature, hypoxia, activity and restricted air access on respiratory physiology of the Alaska blackfish *Dallia pectoralis*. *The Journal of Experimental Biology* **217**, 4387–4398. (doi:10.1242/jeb.105023).

Lefevre, S., Bayley, M. & McKenzie, D.J., 2015 Measuring oxygen uptake in fishes with bimodal respiration. *Journal of Fish Biology* **1**, 1–26.

Lefevre, S. 2016 Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO2 and their interaction. *Conservation Physiology* **4**, 1-31. (doi :10.1093/conphys/cow009).

Lefrançois, C. & Claireaux, G. 2003 Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole Solea solea. *Marine Ecology Progress Series* **259**, 273–284. (doi:10.3354/meps259273).

Leggett, W.C. 1977 The Ecology of fish migrations. *Annual Review of Ecology and Systematics* **8**, 285–308.

Lehtonen, H. 1996 Potential effects of global warming on northern European freshwater fish and fisheries. *Fisheries Management and Ecology* **3**, 59–71.

Lennox, R., Choi, K., Harrison, P.M., Paterson, J.E., Peat, T.B., Ward, T.D., & Cooke, S.J. 2015 Improving science-based invasive species management with physiological knowledge, concepts, and tools. *Biological Invasions*. (doi:10.1007/s10530-015-0884-5).

Lenz, M., da Gama, B.A.P., Gerner, N.V., Gobin, J., Gröner, F., Harry, A. et al. 2011 Non-native marine invertebrates are more tolerant towards environmental stress than taxonomically related native species: results from a globally replicated study. *Environmental Research* **111**, 943–952. (doi:10.1016/j.envres.2011.05.001).

Levins, R. 1962 Theory of fitness in a heterogeneous environment. I. The fitness set and adaptive function. *The American Naturalist* **96**, 361-373.

Lockwood, J.L., Cassey, P. & Blackburn, T. 2005 The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution* **20**, 223–228. (doi:10.1016/j.tree.2005.02.004).

Lockwood, J.L.F., Hoopes, M.F. & Marchetti, M.P., 2013 Invasion ecology. West Sussex, John Wiley & Sons, Ltd.

Lopez, D.P., Jungman, A.A. & Rehage, J.S. 2012 Nonnative African jewelfish are more fit but not bolder at the invasion front: a trait comparison across an Everglades range expansion. *Biological Invasions* **14**, 2159–2174.

Lovegrove, B.G. 2000 The Zoogeography of Mammalian Basal Metabolic Rate. *The American Naturalist* **156**, 201–219.

Maazouzi, C., Piscart, C., Legier, F., & Hervant, F. 2011 Ecophysiological responses to temperature of the “killer shrimp” Dikerogammarus villosus: is the invader really stronger than the native Gammarus pulex? *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **159**, 268–274. (doi:10.1016/j.cbpa.2011.03.019).

Maitland, P.S. & Campbell, R.N. 1992 Freshwater fishes. Glasgow, Harper Collins, pp.369.

Maitland, P.S. 2007 Scotland’s Freshwater Fish: Ecology, Conservation & Folklore. Trafford Publishing.

Malloy, K.D. & Targett, T.E. 1994 Effects of ration limitation and low temperature on growth, biochemical condition, and survival of juvenile summer flounder from two Atlantic coast nurseries. *Transactions of the American Fisheries Society* **123**, 182-193.

Mandic, M., Sloman, K.A & Richards, J.G. 2009a Escaping to the surface: a phylogenetically independent analysis of hypoxia-induced respiratory behaviors in sculpins. *Physiological and Biochemical Zoology* **82**, 730–738. (doi:10.1086/605932).

Mandic, M., Todgham, A.E. & Richards, J.G. 2009b Mechanisms and evolution of hypoxia tolerance in fish. *Proceedings Biological Sciences / The Royal Society* **276**, 735–744. (doi:10.1098/rspb.2008.1235).

Mandic, M., Speers-Roesch, B. & Richards, J.G. 2013 Hypoxia tolerance in sculpins is associated with high anaerobic enzyme activity in brain but not in liver or muscle. *Physiological and Biochemical Zoology* **86**, 92–105. (doi:10.1086/667938).

Mann, R.H.K. 1989 Fish population dynamics in the river Frome, Dorset. *Reg. Rivers. Res. Manag.***4**, 165-177.

Marchetti, M.P., Moyle, P.B., & Levine, R. 2004 Invasive species profiling Exploring the characteristics of non-native fishes acr. *Freshwater Biology* **49**, 646–661. (doi:10.1111/j.1365-2427.2004.01202.x).

Marras, S., Cucco, A., Antognarelli, F., Azzurro, E., Milazzo, M., Bariche, M., et al. 2015 Predicting future thermal habitat suitability of competing native and invasive fish species: from metabolic scope to oceanographic modelling. *Conservation Physiology* **3**, 1–14. (doi:10.1093/conphys/cou059).

McColl D., Yeomans W.E., McGillivray C. & Olszewska J.P. 2009 The River Clyde Fishery Management Plan 2009-2015. Clyde River Foundation, Report 2009/09, 82pp.

McDowall, R.M. 2006. Crying wolf, crying foul, or crying shame: alien salmonids and a biodiversity crisis in the south- ern cool-temperate galaxioid fishes? *Reviews in Fish Biology and Fisheries* **16**, 233–422.

McHugh, P. & Budy, P. 2005 An experimental evaluation of competitive and thermal effects on brown trout (Salmo trutta) and Bonneville cutthroat trout (Oncorhynchus clarkii utah) performance along an altitudinal gradient. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 2784–2795. (doi:10.1139/F05-184).

Metcalfe, N.B., Taylor, A.C. & Thorpe, J.E. 1995 Metabolic rate, social status and life-history strategies in Atlantic salmon. *Animal Behaviour* **49**, 431–436.

Metcalfe, N.B., Van Leeuwen, T.E. & Killen, S.S. 2016a Does individual variation in metabolic phenotype predict fish behaviour and performance? *Journal of Fish Biology* **88**, 298–321. (doi :10.1111/jfb.12699).

Metcalfe, J.D., Wright, S., Tudorache, C. & Wilson, R.P. 2016b Recent advances in telemetry for estimating the energy metabolism of wild fishes. *Journal of Fish Biology* **88**, 284–297. (doi:10.1111/jfb.12804).

Merriam CH. 1894. Laws of temperature control of the geographic distribution of terrestrial animals and plants. Natl. Geogr.Mag. **6**, 229–238

Michonneau, F., Brown, J.W. & Winter, D. 2015 rotl, an R package to interact with the Open Tree of Life data. (PeerJ PrePrints).

Mills, M.D., Rader, R.B. & Belk, M.C. 2004 Complex interactions between native and invasive fish: the simultaneous effects of multiple negative interactions. *Oecologia* **141**, 713–721.

Miller, R.S. 1967 Pattern and Process in Competition. Advances in Ecological Research (Vol. 4). Elsevier Volume 4, Pages iii-viii, 1-311 by Cragg J.B. (ed.).

Morosawa, T. 2011 Hypoxia tolerance of three native and three alien species of bitterling inhabiting Lake Kasumigaura, Japan. *Environmental Biology of Fishes* **91**, 145–153. (doi:10.1007/s10641-011-9767-5).

Mortensen, A., Ugedal, O. & Lund, F. 2007 Seasonal variation in the temperature preference of Arctic charr (Salvelinus alpinus). *Journal of Thermal Biology* **32**, 314–320. (doi:10.1016/j.jtherbio.2007.03.004).

Moyle P.B. 1986 Fish introductions into North America: patterns and ecological impact. In: Mooney HA and Drake JA (eds) Ecology of Biological Invasions of North America and Hawaii, pp 27–43. Springer-Verlag, New York.

Munday, P.L., Donelson, J.M. & Domingos, J.A. 2016 Potential for adaptation to climate change in a coral reef fish. *Global Change Biology*. (doi:10.1111/gcb.13419).

Murchie, K.J., Cooke, S.J., Danylchuk, A.J. & Suski, C.D. 2011 Estimates of field activity and metabolic rates of bonefish (Albula vulpes) in coastal marine habitats using acoustic tri-axial accelerometer transmitters and intermittent-flow respirometry. *Journal of Experimental Marine Biology and Ecology* **396**, 147–155. (doi:10.1016/j.jembe.2010.10.019).

Myles-Gonzalez, E., Burness, G., Yavno, S., Rooke, A. & Fox, M.G. 2015 To boldly go where no goby has gone before: Boldness, dispersal tendency, and metabolism at the invasion front. *Behavioral Ecology* **26**, 1083–1090. (doi:10.1093/beheco/arv050).

Nakagawa, S. & Schielzeth, H. 2013 A general and simple method for obtaining $R^2$ from generalized linear mixed-effects models. *Methods in Ecology and Evolution* **4**, 133-142.

Nakayama, S., Laskowski, K.L., Klefoth, T. & Arlinghaus, R. 2016 Between- and within-individual variation in activity increases with water temperature in wild perch. *Behavioral Ecology* **00**, 1-8. (doi:10.1093/beheco/arw090).

Naya, D.E., Spangenberg, L., Naya, H. & Bozinovic, F. 2012. Latitudinal Patterns in Rodent Metabolic Flexibility. *The American Naturalist* **179**, 172–179. (doi:10.1086/665646).

Naya, D. & Bozinovic, F. 2012. Metabolic scope of fish species increases with distributional range. *Evolutionary Ecology Research* **14**, 1–9.

Near, T.J., Dornburg, A., Eytan, R.I., Keck, B.P., Smith, W.L., Kuhn, K.L., et al. 2013 Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. *Proceedings of the National Academy of Sciences* **110**, 12738–12743. (doi:10.5061/dryad.d3mb4).

Nilsson, G. E. (2007). Gill remodeling in fish--a new fashion or an ancient secret? *The Journal of Experimental Biology **210***, 2403–2409. (doi:10.1242/jeb.000281).

Norin, T. & Malte, H. 2012 Intraspecific variation in aerobic metabolic rate of fish: relations with organ size and enzyme activity in brown trout. *Physiological and Biochemical Zoology* **85**, 645-656.

Norin, T., Malte, H. & Clark, T.D. 2014 Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *The Journal of Experimental Biology* **217**, 244–251. (doi:10.1242/jeb.089755).

Norin, T. & Clark, T.D. 2016 Measurement and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology* **88**(**1**), 122–151.

Nunn, A.D., Copp, G.H., Vilizzi, L. & Carter, M. G. 2010 Seasonal and diel patterns in the migrations of fishes between a river and a floodplain tributary. *Ecology of Freshwater Fish* **19**, 153–162. (doi:10.1111/j.1600-0633.2009.00399.x).

Ojanguren, A.F., Reyes-Gavilán, F.G. & Braña, F. 2001 Thermal sensitivity of growth, food intake and activity of juvenile brown trout. *Journal of Thermal Biology* **26**, 165-170.

Oyugi, D. O., Cucherousset, J. & Robert Britton, J. 2011 Temperature-dependent feeding interactions between two invasive fishes competing through interference and exploitation. *Reviews in Fish Biology and Fisheries* **22**, 499–508. (doi:10.1007/s11160-011-9243-5).

Orme, D. 2013 The caper package: comparative analysis of phylogenetics and evolution in R. *R package version* **5**.

Overgaard, J., Kearney, M.R. & Hoffmann, A.A. 2014 Sensitivity to thermal extremes in Australian Drosophila implies similar impacts of climate change on the distribution of widespread and tropical species. *Global Change Biology*, 1–13. (doi:10.1111/gcb.12521).

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J.K., Thomas, C.D., Descimon, H., et al. 1999 Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* **399**, 579–583. (doi:10.1038/21181).

Payne, N.L., Smith, J.A., Meulen, D.E., Taylor, M.D., Watanabe, Y.Y., Takahashi, A., Marzullo, T.A., Gray, C.A., Cadiou, G. & Suthers, I.M. 2016 Temperature dependence of fish performance in the wild: links with species biogeography and physiological thermal tolerance. *Functional Ecology* **30**, 903-912.

Pecl, K. 1990 The illustrated guide to fishes of lakes and rivers (translated from Czech by Krojzl C & Krojzl J). Artia, Prague, 233pp.

Perry, A.L., Low, P.J., Ellis, J.R., & Reynolds, J.D. 2005 Climate change and distribution shifts in marine fishes. *Science* **308**, 1912–1915. (doi.org/10.1126/science.1111322).

Pimentel, D., Zuniga, R. & Morrison, D. 2005 Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* **52**, 273–288. (doi:10.1016/j.ecolecon.2004.10.002).

Poff, N.L., Brinson, M.M. & Day, J.W. 2002. Aquatic Ecosystems & Global Climate Change – Potential Impacts on Inland Freshwater and Coastal Wetland Ecosystems in the United States. Arlington, VA: Pew Center on Global Climate Change.

Poloczanska, E.S., Brown C.J., Sydeman W.J., Kiessling W., Schoeman D.S., Moore P.J., Brander K., et al. 2013. Global Imprint of Climate Change on Marine Life. *Nature Climate Change* **3**. 1–7. (doi:10.1038/nclimate1958).

Poloczanska, E.S, Burrows M.T, Brown C.J., Molinos J.G., Halpern B.S., Hoegh-Guldberg O., Kappel C.V., et al. 2016. Responses of Marine Organisms to Climate Change across Oceans. *Frontiers in Marine Science* **3,** 1-21. (doi:10.3389/fmars.2016.00062).

Pörtner, H.O. 2001 Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**, 137-146.

Pörtner, H.O. & Knust, R. 2007 Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315**, 95–97.
Pörtner, H.O. & Farrell, A. 2008 Physiology and climate change. *Science* **332**, 690–692.

Pörtner, H.O. & Giomi, F. 2013 Nothing in experimental biology makes sense except in the light of ecology and evolution - correspondence on *Journal of Experimental Biology* **216**, 2771-2782. *Journal of Experimental Biology* **216**, 4494-4495.

Pörtner, H.O. 2014 How and how not to investigate the oxygen and capacity limitation of thermal tolerance (OCLTT) and aerobic scope – remarks on the article by Gräns et al. *Journal of Experimental Biology* **217**, 4432-4433.

Pörtner, H.O. & Gutt, J. 2016 Impacts of climate variability and change on (marine) animals: Physiological underpinnings and evolutionary consequences. *Integrative and Comparative Biology* **56**(**1**), 31–44.

Prenda, J., Rossomanno, S. & Armitage, P.D. 1997a Species interactions and substrate preferences in three small benthic fishes. *Limnetica* **13**, 47-53.

Prenda, J., Armitage, P.D. & Grayston, A. 1997b Habitat use by the fish assemblages of two chalk streams. *Journal of Fish Biology* **51**, 64-79.

Prosser, C.L. 1991 Comparative animal physiology, environmental and metabolic animal physiology, John Wiley & Sons. Hoboken, NJ, USA.

Purchase, C.F. & Brown, J.A. 2000 Interpopulation differences in growth rates and food conversion efficiencies of young Grand Banks and Gulf of Maine Atlantic cod (Gadus morhua). *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 2223-2229.

Rahel, F.J. & Olden, J.D. 2008) Assessing the effects of climate change on aquatic invasive species. *Conservation Biology* **22**, 521–533.

Regan C.T. 1911. The freshwater fishes of the British Isles. London.

Rehage, J. & Sih, A. 2004 Dispersal behavior, boldness, and the link to invasiveness: a comparison of four Gambusia species. *Biological Invasions* **6**, 379–391.

Reidy, S.P., Nelson, J.A., Tang, Y. & Kerr, S.R. 1995 Post-exercise metabolic rate in Atlantic cod and its dependence upon the method of exhaustion. *Journal of Fish Biology* **477**, 377–386. (doi:10.1111/j.1095-8649.1995.tb01907.x).

Reid, D., Armstrong, J.D. & Metcalfe, N.B. 2012 The performance advantage of a high resting metabolic rate in juvenile salmon is habitat dependent. *The Journal of Animal Ecology* **81**, 868–875. (doi:10.1111/j.1365-2656.2012.01969.x).

Rezende, E.L, Bozinovic, F. & Garland, T. 2004 Climatic Adaptation and the Evolution of Basal and Maximum Rates of Metabolism in Rodents. *Evolution* **58**, 1361–1374.

Rezende, E.L., Silva-Durán, I., Novoa, F.F. & Rosenmann, M. 2001 Does thermal history affect metabolic plasticity?: a study in three Phyllotis species along an altitudinal gradient. *Journal of Thermal Biology* **26**, 103–108.

Ribeiro, F., Elvira, B., Collares-Pereira, M.J. & Moyle, P.B. 2008 Life-history traits of non-native fishes in Iberian watersheds across several invasion stages: A first approach. *Biological Invasions* **10**, 89–102. (doi:10.1007/s10530-007-9112-2).

Ricciardi, A., 2007 Are modern biological invasions an unprecedented form of global change? *Conservation Biology* **21**(**2**), 329–336.

Rummer, J.L., Couturier, C.S., Stecyk, J.A.W., Gardiner, N.M., Kinch, J.P., Nilsson, G.E. & Munday, P.L. 2013 Life on the edge: thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology*, 1-12. (doi:10.1111/gcb.12455).

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, M., Molofsky, J. et al. 2001 The Population Biology of Invasive Species. *Annual Review of Ecology and Systematics*, *32*, 305–332. (doi:10.1146/annurev.ecolsys.32.081501.114037).

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E. & Bloomfield, J. 2000 Global Biodiversity Scenarios for the Year 2100. *Science* **287**, 1770–1774. (doi:10.1126/science.287.5459.1770).

Schurmann H. & Steffensen J.F. 1997 Effects of temperature, hypoxia and activity on the metabolism of Atlantic cod, *Gadus morhua*. *Journal of Fish Biology* **50**, 1166-1180.

Seebacher, F., Brand, M.D., Else, P.L., Guderley, H., Hulbert, A.J. & Moyes, C.D. 2010 Plasticity of oxidative metabolism in variable climates: molecular mechanisms. *Physiological and Biochemical Zoology* **83**, 721-732

Seebacher, F., Ward, A.J.W. & Wilson, R.S. 2013 Increased aggression during pregnancy comes at a higher metabolic cost. *Journal of Experimental Biology* **216**, 771–776.

Seebacher, F., White, C.R. & Franklin, C.E. 2015 Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change* **5**, 61–66. (doi:10.1038/nclimate2457).

Selong, J.H., McMahon, T.E., Zale, A.V. & Barrows, F.T. 2001 Effect of temperature on growth and survival of bull trout, with application of an improved method for determining thermal tolerance in fishes. *Transactions of the American Fisheries Society* **130**, 1026-1037.

Speers-Roesch, B., Mandic, M., Groom, D.J.E. & Richards, J.G. 2013 Critical oxygen tensions as predictors of hypoxia tolerance and tissue metabolic responses during hypoxia exposure in fishes. *Journal of Experimental Marine Biology and Ecology* **449**, 239–249. (doi:10.1016/j.jembe.2013.10.006).

Seth, H., Gräns, A., Sandblom, E., Olsson, C., Wiklander, K., Johnsson, J.I. & Axelsson M. 2013 Metabolic Scope and Interspecific Competition in Sculpins of Greenland Are Influenced by Increased Temperatures Due to Climate Change. *PloS One* **8**, 1-6.

Sharma, S., Jackson, D., Minns, C.K. & Shuter, B.J. 2007 Will northern fish populations be in hot water because of climate change? *Global Change Biology* **13**, 2052–2064.

Sims, D.W., Wearmouth, V.J., Genner, M.J., Southward, A.J. & Hawkins, S.J. 2004 Low-temperature-driven early spawning migration of a temperate marine fish. *Journal of Animal Ecology* **73**, 333–341. (doi:10.1111/j.0021-8790.2004.00810.x).

Smith, I.P. & Taylor, A.C. 1993 The energetic cost of agonistic behaviour in the velvet swimming crab, Necora (= Liocarcinus) puber (L.). *Animal Behaviour **45***, 375-391. (doi:10.1006/anbe.1993.1042).

Somero, G.N. 2005 Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. *Frontiers in Zoology* **2**, 1-9. (doi:10.1186/1742-9994-2-1)

Somero, G.N. 2011 Comparative physiology: a "crystal ball" for predicting consequences of global change. *American journal of physiology. Regulatory, integrative and comparative physiology* **301**, R1–R14. (doi: 10.1152/ajpregu.00719.2010).

Steffensen, J.F. 1989 Some errors in respirometry of aquatic breathers. *Fish Physiology and Biochemistry* **6**, 49-59.

Stevens, E.D. & Fry, F.E.J. 1974 Heat transfer and body temperatures in non-thermoregulatory teleosts. *Canadian Journal of Zoology* **52**, 1137–1143

Sunday, J.M., Bates, A.E. & Dulvy, N.K. 2011 Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings. Biological sciences / The Royal Society* **278**, 1823–1830. (doi:10.1098/rspb.2010.1295).

Sunday, J.M., Bates, A.E. & Dulvy, N.K. 2012 Thermal tolerance and the global redistribution of animals. *Nature Climate Change* **2**, 686–690. (doi:10.1038/nclimate1539).

Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T. & Huey, R.B. 2014 Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences* **111**, 5610–5561. (doi:10.1073/pnas.1316145111).

Snyder, W.E. & Evans, E.W. 2006 Ecological effects of invasive arthropod generalist predators. *Annual Review of Ecology and Systematics* **37**, 95–122.

Smyly, W.J.P. 1955 On the biology of stone loach Neamcheilus barbatula. *Journal of Animal Ecology* **24**, 167–186.

Smyly, W.J.P. 1957 The life-history of the bullhead or miller's thumb (*Cottus gobio* L.). *Proceedings of Zoological Society of London* **128**, 431-453.

Taniguchi, Y., Rahel, F.J., Novinger, D.C. & Gerow, K.G. 1998 Temperature mediation of competitive interactions among three fish species that replace each other along longitudinal stream gradients. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 1894–1901. (doi:10.1139/f98-072).

Tomlinson ML, Perrow MR. 2003. Ecology of the Bullhead Cottus gobio, Conserving Natura Rivers Ecology Series No. 4. English Nature: Peterborough; 16.

Tomlinson, S., Dixon, K.W., Didham, R.K. & Bradshaw, S.D. 2015 Physiological plasticity of metabolic rates in the invasive honey bee and an endemic Australian bee species. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* **185**, 835-844. (doi:10.1007/s00360-015-0930-8).

Tudorache, C., Viaene, P., Blust, R., Vereecken, H. & De Boeck, G. 2008 A comparison of swimming capacity and energy use in seven European freshwater fish species. *Ecology of Freshwater Fish* **17**, 284–291. (doi:10.1111/j.1600-0633.2007.00280.x).

Virkkala, R. & Lehikoinen, A. 2014 Patterns of climate-induced density shifts of species: poleward shifts faster in northern boreal birds than in southern birds. *Global Change Biology* 1–9. (doi:10.1111/gcb.12573).

Wang, X.Z., Gan, X.N., Li, J.B., Mayden, R.L. & He, S.P. 2012 Cyprinid phylogeny based on Bayesian and maximum likelihood analyses of partitioned data: Implications for Cyprinidae systematics. *Science China Life Sciences* **55**, 761–773. (doi:10.1007/s11427-012-4366-z).

Wang, T., Lefevre, S., Iversen, N.K., Findorf, I., Buchanan, R. & McKenzie, D.J. 2014 Anaemia only causes a small reduction in the upper critical temperature of sea bass: is oxygen delivery the limiting factor for tolerance of acute warming in fishes? *Journal of Experimental Biology* **217**, 4275-4278.

Warner, S.C., Travis, J. & Dunson, W.A. 1993 Effect of pH Variation of Interspecific Competition Between Two Species of Hylid Tadpoles. *Ecology* **74**, 183–194.

Welcomme, R.L. 1991 International introductions of freshwater fish species into Europe. *Finn. Fish. Res.* 12, 11–18.

Welcomme, R. L. 1992. A history of international introductions of inland aquatic species. ICES Marine Science Symposia **194**, 3–14.

Welcomme RL (1998) International introductions of inland aquatic species. FAO, Rome

Welton, J.S., Mills, C.A. & Rendle, E.L. 1983 Food and habitat partitioning in two small benthic fishes, Noemacheilus barbatula (L.) and Cottus gobio L. *Arch. Hydrobiol.* **97**, 434-454.

Welton, J.S., Mills, C.A. & Pygott, J.R. 1991 The effect of interaction betweenn the stone loach Noemacheilus barbatula (L.) and the bullhead Cottus gobio (L.) on prey and habitat selection. Hydrobiology **200**, 1-7.

Whiteley, N.M., Rastrick, S.P.S., Lunt, D.H. & Rock, J. 2011 Latitudinal variations in the physiology of marine gammarid amphipods. *Journal of Experimental Marine Biology and Ecology* **400**, 70–77. (doi:10.1016/j.jembe.2011.02.027).

Wheeler,A. 1969. The fishes of British Isles and N.W. Europe. Macmillan, London, 537pp.

Williams, F. et al. 2010 The Economic Cost of Invasive Non-Native Species on Great Britain, 1–199.

Williams, R., Pernetta, A.P. & Horrocks, J.A. 2016 Outcompeted by an invader? Interference and exploitative competition between tropical house gecko (Hemidactylus mabouia) and Barbados leaf-toed gecko (Phyllodactylus pulcher) for diurnal refuges in anthropogenic coastal habitats. *Integrative Zoology* **11**, 229–238. (doi:10.1111/1749-4877.12194).

Williamson, M.H. & Fitter, A. 1996 The characters of successful invaders. *Biological Conservation* **78,** 163–170.

Williamson, M. H., 1996 Biological invasions. Population and community biology series, Vol. 15. Chapman and Hall, London. 244 p.

Wilson, R.S., Franklin, C.E., Davison, W. & Kraft, P. 2001 Stenotherms at sub-zero temperatures: thermal dependence of swimming performance in Antarctic fish. *Journal of Comparative Physiology B* **171**, 263-269.

Van Dijk, P.L.M., Staaks, G. & Hardewig, I. 2002 The effect of fasting and refeeding on temperature preference, activity and growth of roach, Rutilus rutilus. *Oecologia* **130**, 496–504. (doi:10.1007/s00442-001-0830-3).

Yang, L., Sado, T., Vincent Hirt, M., Pasco-Viel, E., Arunachalam, M., Li, J., et al. 2015 Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution* **85**, 97–116. (doi:10.1016/j.ympev.2015.01.014).

Yeomans, W.E. & Jennings G. 2013 The River Clyde Fishery Management Plan Review 2013. Clyde River Foundation.

Zeng, L.Q., Fu, S.J. & Cao, Z.D. 2016 Correlations between Standard Metabolic Rate and Metabolic Characteristics after Exhaustive Exercise, and Behavior in the Juvenile Crucian Carp (*Carassius auratus*). *Acta Ecologica Sinica 生态学报* **36** (**6**), 1746–1755.

Zimmerman, J.K.H. & Vondracek, B. 2006 Interactions of slimy sculpin (Cottus cognatus ) with native and nonnative trout : consequences for growth, *Canadian Journal of Fisheries and Aquatic Sciences* **1535**, 1526–1535. (doi:10.1139/F06-054)

## Reference table for studies used for Chapter 2

| Species name | Reference |
|---|---|
| Dallia_pectoralis | Lefevre, S. et al. 2014 Air breathing in the Arctic: influence of temperature, hypoxia, activity and restricted air access on respiratory physiology of the Alaska blackfish Dallia pectoralis. *The Journal of Experimental Biology* **217**,4387–4398. |
| Gymnocanthus_tricuspis | Seth, H., Gräns, A., Sandblom, E., Olsson, C., Wiklander, K., Johnsson, J.I., Axelsson, M. 2013 Metabolic scope and interspecific competition in sculpins of greenland are influenced by increased temperatures due to climate change. *PLoS ONE* **8** e62860. |
| Myoxocephalus_scorpioides | Seth, H., Gräns, A., Sandblom, E., Olsson, C., Wiklander, K., Johnsson, J.I., Axelsson, M. 2013 Metabolic scope and interspecific competition in sculpins of greenland are influenced by increased temperatures due to climate change. *PLoS ONE* **8** e62860. |

| | |
|---|---|
| Myoxocephalus_scorpius | Killen, S.S. et al. 2007 Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings. Biological sciences / The Royal Society* **274**, 431–438. |
| Salvelinus_alpinus | Beamish 1980 |
| Ammodytes_tobianus | Behrens, J.W. et al. 2007 Oxygen dynamics around buried lesser sandeels Ammodytes tobianus (Linnaeus 1785): mode of ventilation and oxygen requirements. *The Journal of Experimental Biology* **210**(**6**), 1006–1114. |
| Anarhichas_lupus | Liao, Y.Y. & Lucas, M.C., 2000. Growth, diet and metabolism of common wolf-fish in the North Sea, a fast-growing population. *Journal of Fish Biology* **56**(**4**), 810–825. |
| Anguilla_anguilla | McKenzie, D.J. et al 2003 Tolerance of chronic hypercapnia by the European eel Anguilla anguilla. *Journal of Experimental Biology* **206**(**10**), 1717–1726. |
| Anguilla_rostrata | Holmberg and Saunders 1979 |
| Aristichthys_nobilis | Yan, G.-J. et al. 2013 An interspecific comparison between morphology and swimming performance in cyprinids. *Journal of Evolutionary Biology* **26**(**8**), 1802–1815. |
| Barbatula_barbatula | Nati unpublished |
| Carassius_auratus | Saunders, R.L., 1962 The irrigation of the gills in fishes. *Canadian Journal of Zoology*, **40**. |
| Carassius_carassius | Fu, S.-J. et al. 2009 The behavioural, digestive and metabolic characteristics of fishes with different foraging strategies. *The Journal of Experimental Biology* **212**(**14**), 2296–2302. |
| Catostomus_commersonii | Saunders, R.L., 1962 The irrigation of the gills in fishes. *Canadian Journal of Zoology*, **40**. |
| Cottus_gobio | Nati unpublished |
| Ctenopharyngodon_idellus | Fu, S.-J. et al. 2009 The behavioural, digestive and metabolic characteristics of fishes with different foraging strategies. *The Journal of Experimental Biology* **212**(**14**), 2296–2302. |
| Cyclopterus_lumpus | Killen, S.S. et al. 2007.Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings. Biological sciences / The Royal Society* **274**, 431–438. |
| Cyprinus_carpio | Pang, X., Cao, Z.-D. & Fu, S.-J. 2011 The effects of temperature on metabolic |

| | |
|---|---|
| | interaction between digestion and locomotion in juveniles of three cyprinid fish (Carassius auratus, Cyprinus carpio and Spinibarbus sinensis). *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **159**, 253–260. |
| Dicentrarchus_labrax | Claireaux, G., Couturier, C. & Groison, A.-L. 2006 Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (Dicentrarchus labrax). *The Journal of Experimental Biology* **209**, 3420–3428. |
| Esox_lucius | Armstrong, J.D., Priede, I.G. & Lucas, M.C. 1992 The link between respiratory capacity and changing metabolic demands during growth of northern pike, Esox lucius L. *Journal of Fish Biology* **41**, 65–75. |
| Fundulus_heteroclitus | Healy, T.M. & Schulte, P.M. 2012 Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish (Fundulus heteroclitus). *Physiological and Biochemical Zoology* **85**, 107-120. |
| Gadus_macrocephalus | Hanna, S.K. et al., 2008 Temperature effects on metabolic rate, swimming performance and condition of Pacific cod Gadus macrocephalus Tilesius. *Journal of Fish Biology* **72**, 1068–1078. |
| Gadus_morhua | Claireaux, G. et al. 2000 Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (Gadus morhua ). *Journal of Sea Research* **44**, 257–265. |
| Gadus_ogac | Bushnell, P.G. et al. 1994 Exercise metabolism in two species of cod in arctic waters. *Polar Biology* **14**, 43–48. |
| Gambusia_holbrooki | Seebacher, F., Ward, a. J.W. & Wilson, R.S. 2013 Increased aggression during pregnancy comes at a higher metabolic cost. *Journal of Experimental Biology* **216**, 771–776. |
| Gila_atraria | Rajagopal and Kramer 1974 |
| Ictalurus_nebulosus | Saunders, R.L., 1962 The irrigation of the gills in fishes. *Canadian Journal of Zoology*, **40**. |
| Leiostomus_xanthrus | Horodysky, A.Z. et al. 2011 Comparative metabolic rates of common western North Atlantic Ocean sciaenid fishes. *Journal of Fish Biology* **79**, 235–55. |

| | |
|---|---|
| Lepomis_gibbosus | Brett, J.R. & Sutherland, D.B. 1965 Respiratory Metabolisrn of Purnphinseed ( Lepornis gibbosus ) in Relation to Swirnrning Speed. *Journal of Fish Research BD. Canada* **22**, 0–4. |
| Limanda_limanda | Duthie, G.G. 1982 The respiratory metabolism of temperature-adapted flatfish at rest and during swimming activity and the use of anaerobic metabolism at moderate swimming speeds. *Journal of Experimental Biology* **97**, 359-373 |
| Liza_aurata | Killen, S.S., Reid, D., Marras, S., Domenici, P. 2015 The interplay between aerobic metabolism and antipredator performance: vigilance is related to recovery rate after exercise. *Frontiers in Physiology* **6**. |
| Macrozoraces_americanus | Killen, S.S. et al. 2007.Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings. Biological sciences / The Royal Society* **274**, 431–438. |
| Melanogrammus_aeglefinus | Tytler 1969 |
| Merlangius_merlangus | Steinhausen, M.F., Steffensen, J.F. & Andersen, N.G. 2005 Tail beat frequency as a predictor of swimming speed and oxygen consumption of saithe (Pollachius virens) and whiting (Merlangius merlangus) during forced swimming. *Marine Biology* **148**, 197–204. |
| Micropterus_salmoides | Beamish, F.W.H. 1970 Oxygen consumption of largemouth bass. *Canadian Journal of Zoology* **48**, 1221–1228. |
| Microstomus_kitt | Duthie, G.G. 1982 The respiratory metabolism of temperature-adapted flatfish at rest and during swimming activity and the use of anaerobic metabolism at moderate swimming speeds. *Journal of Experimental Biology* **97**, 359-373 |
| Morone_saxatilis | Lapointe, D. et al. 2014 Temperature, hypoxia, and mycobacteriosis: effects on adult striped bass Morone saxatilis metabolic performance. *Diseases of aquatic organisms* **108**, 113–127. |
| Mylopharodon_conocephalus | Fangue, N. a. et al. 2015 Juvenile and adult hardhead Mylopharodon conocephalus oxygen consumption rates: effects of temperature and swimming velocity. *Environmental Biology of Fishes* **98**, 585–596. |

| | |
|---|---|
| Mylopharyngodon_piceus | Yan, G.-J. et al. 2013 An interspecific comparison between morphology and swimming performance in cyprinids. *Journal of Evolutionary Biology* **26**(**8**), 1802–1815. |
| Oncorhynchus_mykiss | Bushnell et al 1984 |
| Oncorhynchus_nerka | Brett and Glass 1973 |
| Oncorhynchus_gorbuscha | Clark, T.D., Jeffries, K.M., Hinch, S.G., Farrell, A.P. 2011 Exceptional aerobic scope and cardiovascular performance of pink salmon (Oncorhynchus gorbuscha) may underlie resilience in a warming climate. *The Journal of Experimental Biology* **214**, 3074-3081. |
| Parabramis_pekinensis | Peng, J., Cao, Z.-D. & Fu, S.-J. 2014 The effects of constant and diel-fluctuating temperature acclimation on the thermal tolerance, swimming capacity, specific dynamic action and growth performance of juvenile Chinese bream. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology* **176**, 32–40. |
| Phoxinus_phoxinus | Killen unpublished |
| Platichthys_flesus | Duthie, G.G. 1982 The respiratory metabolism of temperature-adapted flatfish at rest and during swimming activity and the use of anaerobic metabolism at moderate swimming speeds. *Journal of Experimental Biology* **97**, 359-373. |
| Pleuronectes_platessa | Priede and Holliday 1980 |
| Pollachius_virens | Steinhausen, M.F., Steffensen, J.F. & Andersen, N.G. 2005 Tail beat frequency as a predictor of swimming speed and oxygen consumption of saithe (Pollachius virens) and whiting (Merlangius merlangus) during forced swimming. *Marine Biology* **148**, 197–204. |
| Polyodon_spathula | Aboagye, D.L. & Allen, P.J., 2014. Metabolic and locomotor responses of juvenile paddlefish Polyodon spathula to hypoxia and temperature. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **169**, 51–59. |
| Pseudorasbora_parva | Yan, G.-J. et al. 2013 An interspecific comparison between morphology and swimming performance in cyprinids. *Journal of Evolutionary Biology* **26**(**8**), 1802–1815. |
| Rhinichthys_osculus | Rajagopal and Kramer 1974 |

| | |
|---|---|
| Salmo_trutta | Norin, T. & Malte, H. 2012 Intraspecific variation in aerobic metabolic rate of fish: relations with organ size and enzyme activity in brown trout. *Physiological and Biochemical Zoology* **85**, 645-656. |
| Salvelinus_fontinalis | Graham, J.M. 1949 SOME EFFECTS OF TF]MPERATURE AND OXYGEN PRB,SSURE ON THE METABOLISM AND ACTIVITY OF THE SPECKLED TROUT. SALVELINUS FONTINALISL. *Canadian Journal of Research* 27, 270–288. |
| Sander_lucioperca | Frisk, M., Skov, P.V. & Steffensen, J.F. 2012 Thermal optimum for pikeperch (Sander lucioperca) and the use of ventilation frequency as a predictor of metabolic rate. *Aquaculture* **324**, 151–157. |
| Scomber_scomberus | Killen et al. unpublished |
| Scophthalmus_maximus | Mallekh, R. & Lagardere, J.P. 2002 Effect of temperature and dissolved oxygen concentration on the metabolic rate of the turbot and the relationship between metabolic scope and feeding demand. *Journal of Fish Biology* **60**(**5**), 1105–1115. |
| Solea_solea | Lefrançois, C. & Claireaux, G., 2003 Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole Solea solea. *Marine Ecology Progress Series* **259**, 273–284. |
| Sparus_aurata | Steinhausen, M.F., Fleng Steffensen, J. & Gerner Andersen, N., 2010. The effects of swimming pattern on the energy use of gilthead seabream ( Sparus aurata L.). *Marine and Freshwater Behaviour and Physiology* **43**, 227–241. |
| Acanthochromis_polyacanthus | Rummer, J.L, Couturier, C.S., Stecyk, J.A.W., Gardiner, N.M., Kinch, J.P., Nilsson, G.E., Munday, P.L. 2014 Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology* **20**, 1055-1068. |
| Albula_vulpes | Murchie, K.J., Cooke, S.J., Danylchuk, A.J. & Suski, C.D. 2011 Estimates of field activity and metabolic rates of bonefish (Albula vulpes) in coastal marine habitats using acoustic tri-axial accelerometer transmitters and intermittent-flow respirometry. *Journal of Experimental Marine Biology and Ecology* **396**, 147–155. (doi:10.1016/j.jembe.2010.10.019). |

| | |
|---|---|
| Argyrosomus_japonicus | Fitzgibbon, Q.P., Strawbridge, A. & Seymour, R.S. 2007 Metabolic scope, swimming performance and the effects of hypoxia in the mulloway, Argyrosomus japonicus (Pisces: Sciaenidae). *Aquaculture*, **270**, 358–368. |
| Cheilodipterus_quinquelineatus | Rummer, J.L, Couturier, C.S., Stecyk, J.A.W., Gardiner, N.M., Kinch, J.P., Nilsson, G.E., Munday, P.L. 2014 Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology* **20**, 1055-1068. |
| Chromis_atripectoralis | Gardiner, N.M., Munday, P.L., Nilsson, G.E. 2010 Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PLoS ONE* **5**, e13299 |
| Danio_rerio | Lucas, M. & Priede, I. 1992 Utilization of metabolic scope in relation to feeding and activity by individual and grouped zebrafish, Brachydanio rerio (Hamilton-Buchanan). *Journal of Fish Biology* **41**, 175–190. |
| Dascyllus_aruanus | Gardiner, N.M., Munday, P.L., Nilsson, G.E. 2010 Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PLoS ONE* **5**, e13299 |
| Dascyllus_melanurus | Rummer, J.L, Couturier, C.S., Stecyk, J.A.W., Gardiner, N.M., Kinch, J.P., Nilsson, G.E., Munday, P.L. 2014 Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology* **20**, 1055-1068. |
| Euthynnus_affinis | Sepulveda, C. & Dickson, K.A. 2000 MAXIMUM SUSTAINABLE SPEEDS AND COST OF SWIMMING IN JUVENILE KAWAKAWA TUNA ( EUTHYNNUS AFFINIS ) AND CHUB MACKEREL ( SCOMBER JAPONICUS ). *Journal of Experimental Biology* **203**, 3089–3101. |
| Hypophthalmichthys_molitrix | Yan, G.-J. et al. 2013 An interspecific comparison between morphology and swimming performance in cyprinids. *Journal of Evolutionary Biology* **26**(**8**), 1802–1815. |
| Katsuwonus_pelamis | Dewar, H. & Graham, J.B. 1994 STUDIES OF TROPICAL TUNA SWIMMING PERFORMANCE IN A |

| | |
|---|---|
| | LARGE WATER TUNNEL. *Journal of Experimental Biology* **192**, 13–31. |
| Kuhlia_sandvicensis | Muir and Niimi 1972 |
| Lates_calcarifer | Norin, T., Malte, H. & Clark, T.D. 2014 Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *The Journal of Experimental Biology* **217**, 244–251. (doi:10.1242/jeb.089755). |
| Micropogonias_undulatus | Horodysky, A.Z. et al. 2011 Comparative metabolic rates of common western North Atlantic Ocean sciaenid fishes. *Journal of Fish Biology* **79**, 235–55. |
| Oreochromis_niloticus | Farmer and Beamish 1969 |
| Ostorhinchus_cyanosoma | Gardiner, N.M., Munday, P.L., Nilsson, G.E. 2010 Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PLoS ONE* **5**, e13299 |
| Pagrus_auratus | Cook, D.G., Wells, R.M.G. & Herbert, N.A. 2011 Anaemia adjusts the aerobic physiology of snapper (Pagrus auratus) and modulates hypoxia avoidance behaviour during oxygen choice presentations. *The Journal of Experimental Biology* **214**, 2927–2934. |
| Poecilia_reticulata | Svendsen, J.C. et al. 2013 Effects of intraspecific variation in reproductive traits, pectoral fin use and burst swimming on metabolic rates and swimming performance in the Trinidadian guppy (Poecilia reticulata). *The Journal of Experimental Biology* **216**, 3564–3574. |
| Pomacentrus_amboinensis | Couturier, C.S. et al. 2013 Species-specific effects of near-future CO2 on the respiratory performance of two tropical prey fish and their predator. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **166**, 482–489. |
| Pomacentrus_moluccensis | Gardiner, N.M., Munday, P.L., Nilsson, G.E. 2010 Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PLoS ONE* **5**, e13299 |
| Pomatomus_saltatrix | Freadman, M.A., 1979. Swimming Energetics of Striped Bass ( Morone Saxatilis ) and Bluefish ( Pomatomus Saltatrix ): Hydrodynamic Correlates of Locomotion and Gill Ventilation. *The Journal of Experimental Biology* **83**, 217–230. |

| | |
|---|---|
| Pseudochromis_fuscus | Couturier, C.S. et al. 2013 Species-specific effects of near-future CO2 on the respiratory performance of two tropical prey fish and their predator. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **166**, 482–489. |
| Sarpa_salpa | Marras, S., Cucco, A., Antognarelli, F., Azzurro, E., Milazzo, M., Bariche, M., Butenschön, M., Kay, S., Di Bitetto, M., Quattrocchi, G. et al. 2015 Predicting future thermal habitat suitability of competing native and invasive fish species: From metabolic scope to oceanographic modelling. *Conservation Physiology* **3**. |
| Scolopsis_bilineata | Roche, D.G. et al. 2013 Finding the best estimates of metabolic rates in a coral reef fish. *The Journal of Experimental Biology* **216**, 2103–2110. |
| Scomber_japonicus | Sepulveda, C. & Dickson, K.A. 2000 MAXIMUM SUSTAINABLE SPEEDS AND COST OF SWIMMING IN JUVENILE KAWAKAWA TUNA ( EUTHYNNUS AFFINIS ) AND CHUB MACKEREL ( SCOMBER JAPONICUS ). *Journal of Experimental Biology* **203**, 3089–3101. |
| Seriola_lalandi | Clark, T.D. & Seymour, R.S. 2006 Cardiorespiratory physiology and swimming energetics of a high-energy-demand teleost, the yellowtail kingfish (Seriola lalandi). *The Journal of Experimental Biology* **209**, 3940–3951. |
| Siganus_rivulatus | Marras, S., Cucco, A., Antognarelli, F., Azzurro, E., Milazzo, M., Bariche, M., Butenschön, M., Kay, S., Di Bitetto, M., Quattrocchi, G. et al. 2015 Predicting future thermal habitat suitability of competing native and invasive fish species: From metabolic scope to oceanographic modelling. *Conservation Physiology* **3**. |
| Thunnus_albacares | Dewar, H. & Graham, J.B. 1994 STUDIES OF TROPICAL TUNA SWIMMING PERFORMANCE IN A LARGE WATER TUNNEL. *Journal of Experimental Biology* **192**, 13–31. |
| Zoramia_leptacantha | Rummer, J.L, Couturier, C.S., Stecyk, J.A.W., Gardiner, N.M., Kinch, J.P., Nilsson, G.E., Munday, P.L. 2014 Life on the edge: Thermal optima for aerobic |

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|---|---|
| | scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology* **20**, 1055-1068. |

## Reference table for studies used for Chapter 3

| Species name | Reference |
|---|---|
| Acanthochromis polyacanthus | Gardiner, N.M., Munday, P.L., Nilsson, G.E. 2010 Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PLoS ONE* **5**, e13299. |
| Albula vulpes | Nowell, L., Brownscombe, J., Gutowsky, L.G., Murchie, K., Suski, C., Danylchuk, A., Shultz, A., Cooke, S. 2015 Swimming energetics and thermal ecology of adult bonefish (albula vulpes): A combined laboratory and field study in eleuthera, the bahamas. *Environmental Biology of Fishes* **98**, 2133-2146. |
| Cheilodipterus quinquelineatus | Rummer, J.L, Couturier, C.S., Stecyk, J.A.W., Gardiner, N.M., Kinch, J.P., Nilsson, G.E., Munday, P.L. 2014 Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology* **20**, 1055-1068. |
| Dascyllus aruanus | Gardiner, N.M., Munday, P.L., Nilsson, G.E. 2010 Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PLoS ONE* **5**, e13299. |
| Dascyllus melanurus | Rummer, J.L, Couturier, C.S., Stecyk, J.A.W., Gardiner, N.M., Kinch, J.P., Nilsson, G.E., Munday, P.L. 2014 Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology* **20**, 1055-1068. |
| Forsterygion lapillum | Khan, J.R. & Herbert, N.A. 2012 The behavioural thermal preference of the common triplefin (forsterygion lapillum) tracks aerobic scope optima at the upper thermal limit of its distribution. *Journal of Thermal Biology* **37,** 118-124. |
| Fundulus heteroclitus | Healy, T.M. & Schulte, P.M. 2012 Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish (fundulus heteroclitus). *Physiological and Biochemical Zoology* **85**, 107-120 |
| Gadus morhua | Tirsgaard, B., Behrens, J.W. & Steffensen, J.F. 2015 The effect of temperature and body size on metabolic scope of activity in juvenile atlantic cod gadus morhua l. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **179**, 89-96. |
| Liza aurata | Killen, S.S., Reid, D., Marras, S., Domenici, P. 2015 The interplay between aerobic metabolism and antipredator performance: vigilance is related to |

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|---|---|
| | recovery rate after exercise. *Frontiers in Physiology* **6**.111.doi: 10.3389/fphys.2015.00111 |
| Microstomus kitt | Duthie, G.G. 1982 The respiratory metabolism of temperature-adapted flatfish at rest and during swimming activity and the use of anaerobic metabolism at moderate swimming speeds. *Journal of Experimental Biology* **97,** 359-373. |
| Myoxocephalus scorpioides | Seth, H., Gräns, A., Sandblom, E., Olsson, C., Wiklander, K., Johnsson, J.I., Axelsson, M. 2013 Metabolic scope and interspecific competition in sculpins of greenland are influenced by increased temperatures due to climate change. *PLoS ONE* **8** e62860. |
| Myoxocephalus scorpius | Seth, H., Gräns, A., Sandblom, E., Olsson, C., Wiklander, K., Johnsson, J.I., Axelsson, M. 2013 Metabolic scope and interspecific competition in sculpins of greenland are influenced by increased temperatures due to climate change. *PLoS ONE* **8** e62860 |
| Oncorhynchus clarkii | Dwyer, W.P. & Kramer, R.H. 1975 The influence of temperature on scope for activity in cutthroat trout, salmo clarki. *Transactions of the American Fisheries Society* **104**, 552-554. |
| Oncorhynchus gorbuscha | Clark, T.D., Jeffries, K.M., Hinch, S.G., Farrell, A.P. 2011 Exceptional aerobic scope and cardiovascular performance of pink salmon (Oncorhynchus gorbuscha) may underlie resilience in a warming climate. *The Journal of Experimental Biology* **214**, 3074-3081. |
| Oncorhynchus kisutch | Lee, C.G., Farrell, A.P., Lotto, A., MacNutt, M., Hinch, S. & Healey, M. 2003 The effect of temperature on swimming performance and oxygen consumption in adult sockeye (Oncorhynchus nerka) and coho (O. kisutch) salmon stocks. *Journal of Experimental Biology* **206**, 3239-3251. |
| Oncorhynchus mykiss | Chen, Z., Snow, M., Lawrence, C.S., Church, A.R., Narum, S.R., Devlin, R.H. & Farrell, A.P. 2015 Selection for upper thermal tolerance in rainbow trout (Oncorhynchus mykiss walbaum). *The Journal of Experimental Biology* **218**, 803-812. |
| Oncorhynchus nerka | Lee, C.G., Farrell, A.P., Lotto, A., MacNutt, M., Hinch, S. & Healey, M. 2003 The effect of temperature on swimming performance and oxygen consumption in adult sockeye (Oncorhynchus nerka) and coho (O. kisutch) salmon stocks. *Journal of Experimental Biology* **206**, 3239-3251. |
| Ostorhinchus cyanosoma | Gardiner, N.M., Munday, P.L., Nilsson, G.E. 2010 Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PLoS ONE* **5**, e13299. |
| Pagothenia borchgrevinki | Lowe CJ, Davison W (2006) Thermal sensitivity of scope for activity in pagothenia borchgrevinki, a |

| | |
|---|---|
| | cryopelagic antarctic nototheniid fish. Polar Biology 29: 971-977 |
| Polyprion oxygeneios | Khan, J.R., Pether, S., Bruce, M., Walker, S.P. & Herbert, N.A. 2014 Optimum temperatures for growth and feed conversion in cultured hapuku (Polyprion oxygeneios) — is there a link to aerobic metabolic scope and final temperature preference? *Aquaculture* **430,** 107-113. |
| Pomacentrus moluccensis | Gardiner, N.M., Munday, P.L., Nilsson, G.E. 2010 Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PLoS ONE* **5**, e13299. |
| Sarpa salpa | Marras, S., Cucco, A., Antognarelli, F., Azzurro, E., Milazzo, M., Bariche, M., Butenschön, M., Kay, S., Di Bitetto, M., Quattrocchi, G. et al. 2015 Predicting future thermal habitat suitability of competing native and invasive fish species: From metabolic scope to oceanographic modelling. *Conservation Physiology* **3**. |
| Salvelinus fontinalis | Graham, J.M. 1949 Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout. Salvelinus fontinalis. *Canadian Journal of Research* **27**, section D |
| Salvelinus namaycush | Kelly, N.I., Burness, G., McDermid, J.L., Wilson, C.C. 2014 Ice age fish in a warming world: minimal variation in thermal acclimation capacity among lake trout (Salvelinus namaycush) populations. *Conservation Physiology* **2**, cou025; doi:10.1093/conphys/cou025. |
| Sander lucioperca | Frisk, M., Skov, P.V. & Steffensen, J.F. 2012 Thermal optimum for pikeperch (Sander lucioperca) and the use of ventilation frequency as a predictor of metabolic rate. *Aquaculture* **324**, 151–157. |
| Scophthalmus maximus | Mallekh, R. & Lagardere, J.P. 2002 Effect of temperature and dissolved oxygen concentration on the metabolic rate of the turbot and the relationship between metabolic scope and feeding demand. *Journal of Fish Biology* **60**(**5**), 1105–1115. |
| Siganus rivulatus | Marras, S., Cucco, A., Antognarelli, F., Azzurro, E., Milazzo, M., Bariche, M., Butenschön, M., Kay, S., Di Bitetto, M., Quattrocchi, G. et al. 2015 Predicting future thermal habitat suitability of competing native and invasive fish species: From metabolic scope to oceanographic modelling. *Conservation Physiology* **3**. |
| Zoramia leptacantha | Rummer, J.L, Couturier, C.S., Stecyk, J.A.W., Gardiner, N.M., Kinch, J.P., Nilsson, G.E., Munday, P.L. 2014 Life on the edge: Thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology* **20**, 1055-1068. |